

**Revised Comments from Members of the Chemical Assessment Advisory Committee
Augmented for the Review of the EPA's Draft IRIS Hexahydro-1,3,5-trinitro-1,3,5-triazine
(RDX) Assessment**

(September, 2016)

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Dr. Hugh Barton

1. Literature search/study selection and evaluation. The section on Literature Search Strategy| Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Literature search, selection, and evaluation steps appear clearly documented and reasonable; no specific comments.

2. Toxicokinetic modeling. In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al., 2012a; Sweeney et al., 2012b).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported?

Yes. EPA re-evaluated the model and the fits to the data. Some options are noted below that are similarly plausible to the choices EPA made.

Do the revised PBPK models adequately represent RDX toxicokinetics?

- Overall, the models reasonably represent RDX TK with limitations noted in the Supplement text.
- The rat data are relatively strong. EPA chose to change the fitted partition coefficients to predicted values for adipose and muscle, substantially misestimating the iv data in the volume of distribution. This issue had been previously discussed by Sweeney and coauthors. Some exploration could be done of) how much impact it would have on the HED with a smaller Vd (fitted lower partition coefficients vs predicted values. At steady state it may modestly lower the HED.
- For the oral route, the different formulations and particles sizes are problematic in that each formulation requires its own absorption parameters.
- The human data are much less strong, so the modeling is adequate but uncertain. One could apply the fitted rat partition coefficients to humans as generally partitioning reflecting nonspecific tissue binding is fairly similar across species.
- The mouse data is considered the least strong, though EPA could re-evaluate whether the Guo et al 1985 total radioactivity data are consistent with the Sweeney et al 2012 data. The early time points of the Guo et al 1985 study would likely be less confounded by metabolite associated radioactivity, so the data could prove informative. The description of the noncompartmental analysis (C-28) is good to provide perspective on the data versus the PBPK modeling.

Are the model assumptions and parameters clearly presented and scientifically supported?

Yes, section C.1.5 was generally clear and well done. The challenges for estimating absorption rate and fraction absorbed for the human model are largely well discussed and illustrated in a figure. Clearance in the human model was shown to be similarly estimated by scaling rat metabolic clearance with a ratio of in vitro values or by fitting the human data sets, supporting that these values were reasonably estimated.

Are the uncertainties in the model appropriately considered and discussed?

The local sensitivity analysis is fine for AUC, but could be done for C_{max}, since it is also considered as a dose metric and will be sensitive to other parameters. Perhaps the biggest uncertainty is using dose metrics for parent compound when it might be metabolites play a major role in the toxicities, which cannot be quantified by sensitivity analysis.

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate?

For neurotoxicity, the choice of dose metric is sufficiently described (pages 2-8 and 2-9). The choice is a reasonable one given less than ideal data about the pharmacokinetic-pharmacodynamic (PKPD) relationship for this endpoint. A PKPD model likely would be driven by concentration in plasma as a surrogate for brain, so the response would not be fully attributed to either the peak (C_{max}) or the average (AUC/time) concentration. Since the POD_{HED} is presented in Table 2-2 for both dose metrics, the options are transparent and the difference is relatively modest in the rat (~30%).

There does not appear to be an explanation for the choice of dose metric for the prostatitis endpoint, though some comments (e.g., AUC considered better estimated than C_{max}) in the discussion for neurotoxicity apply across endpoints. Again the differences in Table 2-2 are modest, and since this is an effect only observed in a chronic study, average daily AUC is a reasonable choice. The effect might be due to either parent compound or metabolite, so that is a limitation of this choice. Text should be added.

The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

Yes, the mouse model is more uncertain as discussed on page 2-9.

2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

There does not appear to be adequate data to support modeling human population variability in PK to estimate a data derived factor to replace the default uncertainty factor.

3a (ii) Nervous system-specific toxicity values (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described.

The nonhuman primate study (Martin and Hart 1974) should be included in the PODs. It has 6 animals/dose group. Text (p 2-3 line 24) discounts it as 3/sex, but the Crouse study is used combining sexes, so this doesn't make sense as a criticism of the NHP study. This is a rare study to have in a highly relevant species and it is inappropriate to leave it out.

Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration?

Dietary exposure is often considered more representative of human exposure as compared to bolus dosing by gavage. However, given the limited information in the Preface about RDX and exposure, it is not clear that human exposure in this case will be more like dietary exposure (i.e., more frequent, smaller exposures) than like gavage (i.e., periodic bolus exposures), so it is not obvious that one should be preferred over the other.

3a (iii) Points of departure for nervous system endpoints (Section 2.1.2). Is the calculation of the HEDs for these studies scientifically supported and clearly described?

A pharmacokinetic-pharmacodynamic (PKPD) model would use concentration as the driver for effects, so the choice between AUC and Cmax is somewhat arbitrary and driven by limitations on available PKPD time course data. The two arguments for AUC (binding to GABA channel and convulsions after repeated doses) do not necessarily discriminate between Cmax and AUC (p2-9). Effects after repeated doses might reflect changes in the concentration-response relationship due to biological changes in the tissues (exemplifying a non-stationary concentration-response model), thus not distinguishing between Cmax or AUC. The major limitation of Cmax is that it is more uncertain due to the uncertainties around absorption parameters and their variability with the formulation of RDX to which one is exposed, so AUC is a reasonable choice.

The mechanistic evidence (p 1-17) indicates that parent compound can bind GABA receptor and brain RDX concentrations related to the effects observed in rats supporting the use of a dose metric related to parent compound.

3b. Kidney and other urogenital system effects

(i) Kidney and other urogenital system hazard (Sections 1.2.2, 1.3.1). Kidney and other urogenital system hazard (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

The kidney effects occur inconsistently in both rats and mice and but appear to be an endpoint to be addressed at least qualitatively more clearly than is currently the case. It should be carefully reviewed if kidney effects are more consistently observed at high doses. This may not be an effect that would determine the RfD, but substituting prostatitis as the only clearly evaluated seems questionable when that effect may be totally unrelated to kidney effects and has been observed only in the single 2 year study in mice that looked at prostate.

The statement (p1-22 line 30) that the prostatitis provides the strongest evidence of urogenital toxicity seems questionable since it is a singular study, though it may be the most useable for quantitative dose-response analysis.

The mechanistic evidence section (p 1-37-1-38) seems highly speculative. It should be deleted retaining a modified version of the last paragraph, which starts with a statement that there are "not studies" that directly inform the mechanism of effect. The executive summary clearly states the MOA is unknown, supporting that this section should be reduced to a paragraph.

(iii) Points of departure for kidney and other urogenital system endpoints (Section 2.1.2). Is the calculation of the HED for this study scientifically supported and clearly described?

HED: It is not clear the extent to which this effect is dependent upon parent versus metabolites, as compared to the neurological effects making the selection of this HED based upon AUC more uncertain but a reasonable choice. The alternative, allometric scaling from administered dose to rats, introduces its own uncertainties.

3b (iv) Uncertainty factors for kidney and other urogenital system endpoints (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Is database UF appropriate for this endpoint? Has EPA considered whether methods for derivation of overall RfD and for target site specific values may need different choices of UFs? Given the choice of a sensitive endpoint observed in a single study over the kidney effects inconsistently observed in multiple species and studies at higher doses, the database uncertainty factor seems questionable, perhaps inappropriate.

3c (iii) Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of the HED for this study scientifically supported and clearly described?

It is not clear the extent to which this effect is dependent upon parent versus metabolites, as compared to the neurological effects. Allometric scaling of mouse dose is the least uncertain of the options in this case given the limitations on data supporting the mouse parameterization of the PBPK model and uncertainty around active form of the compound.

4b. Inhalation reference concentration for effects other than cancer (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK

inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

Lack of data precludes derivation of inhalation RfC.

4d. Inhalation unit risk for cancer (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

Lack of data precludes derivation of inhalation unit risk for cancer.

Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Generally the executive summary is well written, succinct, and clear. Some specific concerns are noted here.

P xxiii line 7 – 9: The first sentence indicates human potential for kidney and urogenital toxicity, which is fine, but indicates this is “based on” increased relative kidney weights and histopathological changes. P 1-24 lines 24-30 indicates inconsistent findings in the subchronic studies and down plays the organ weight findings in the chronic studies so the executive summary is inconsistent with this.

P xxvii line 23 – 25: It is not clear, given the limited information on RDX exposures in the Preface or elsewhere in the document, that dietary exposure is “more representative of potential human exposures”. In this case it seems possible that human exposures could involve different “formulations” of RDX (e.g., on swallowed dust particles, consumed soil, incorporated into plants) such that neither experimental exposures as diet or as gavage would be obviously “representative” thus there would be uncertainty.

Editorial comments

P 2-22 line 5: "subchronic to chronic duration" UF was not used, rather database uncertainty was.

Supplement Appendix C

C-6 lines 35-37: Note that in addition to the limited evidence for high fat:blood partition coefficients, modeling using these values for the rat intravenous plasma data substantially over estimates the distribution (thus, underestimating plasma concentrations) phase.

Table C-1: Is footnote symbol "d" correct for second row (swine) of brain column?

C-12 line 11: Is PS value 1 correct? Units are incorrect.

C-20 line 7: Change "An alternative to varying the KAS" to "An additional factor along with varying KAS" In the data (Fig C-4), it is difficult to distinguish absorption rate from fraction absorbed and getting through the gut (f_{afg}). (Note, that "bioavailability" is a bit misleading in that I'm assuming the liver metabolism isn't being modified, so what is likely being varied is f_{afg}.) However, KAS will modify the time to peak concentration, while varying f_{afg} will raise or lower the plasma concentration, so these two parameters can have different effects despite the fact that the data in Fig C-4 do not allow one to distinguish.

C-20 line 19: "...provide a more robust estimated parameter." What parameter? More robust than what?

Dr. Marteen Bosland

. Literature search/study selection and evaluation. The section on Literature Search Strategy / Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and mostly clearly described. In Table LS-1 under exposure, "exposure to a mixture only" is listed as exclusion criterion. Because RDX contains contaminants and is therefore a mixture itself, it might be clearer to rephrase this as "exposure to a mixture containing RDX only".

3.b.i. Kidney and other urogenital system hazard (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

The EPA relies almost entirely on the report by Levine et al. (1983) about chronic exposure of F344 rats to RDX for its evaluation of effects on the kidney and urogenital tract and prostatic suppurative inflammation is selected as a "surrogate marker" for these effects.

In the Levine et al. report, prostatic inflammation is categorized into several subtypes, one of which is suppurative inflammation. The other categories include subacute inflammation, chronic-active inflammation, and microabscesses. Reference is made in the EPA assessment document to a paper by Suwa et al. (2001) about background pathology in the prostate of aged F344 rats to conclude that inflammation in the control group of the study by Levine et al. (1983) may have been unusually low for this strain of rats. In the paper by Suwa et al. (2001), which described prostate pathology of 1,768 control F344 rats allowed to live for up to 2.2-2.5 years, all types of inflammation are lumped together and 70.4% of these rats had inflammation, mostly confined to the dorsolateral prostate and mostly graded as mild. No data are provided specifically on suppurative inflammation.

If one does the same lumping for the 24 months groups in the Levine et al. (1983) study, 23/55 control rats (42%) had prostatic inflammation of any type (chronic and suppurative inflammation) and the incidence in the 0.3, 1.5, and 8.0 mg/kg-day dose groups was 20/55 (36%), 22/52 (44%), and 23/55 (42%), respectively; these lesions were mostly graded as minimal-mild. Two of 3 rats that died between 6 and 12 months in the 1.5 mg/kg-day group also had prostatic inflammation, as did 1/10 rats in this dose group and 2/10 rats in the 8.0 mg/kg-day group at the scheduled 12 months interim time-point; the lesions in these animals were all of the (sub)acute inflammatory type. Thus, the incidence of all types of inflammation was similar in the control group and the 0.3, 1.5, and 8.0 mg/kg-day groups, and it was about 40% lower than the incidences reported by Suwa et al. (2001) for aged F344 rats.

In the highest dose group, 24 of 55 rats died before the end of the two year study, 19 of them between 12 and 24 months on study. Prostatic inflammation (chronic type) was found in one of five highest dose rats that died before 6 months and in 18/19 (95%) highest dose rats that died between 6 and 12 months. Suppurative inflammation (and in one rat chronic inflammation) was found in 19/27 (70%) highest dose rats that died between 12 and 24 months. One of the 4 highest dose rats that lived until the end of the study had minimal chronic inflammation; no suppurative prostatic lesions were found in these 4 rats. Thus, the total incidence of prostatic inflammation (of any type) in the 55 rats of the 24 months study was 39/55 (71%). Twenty of the 31 rats (65%) that died after the 12 month time-point (including the 4 that survived until the end of study) had prostatic inflammation (of any type).

As indicated in the EPA assessment document, there is no information in the Levine et al. (1983) report about details of the histopathology methods. It is not clear how the prostate was grossed for histology. The fact that the incidence of prostatic inflammation in the control group was 40% lower than the range of inflammation incidences found in the dorsolateral prostate by Suwa et al. (2001) would suggest that some or many of the prostates examined by Levine et al. were ventral lobes, which have a low inflammation incidence (4-12%) according to Suwa et al. (2001). The Suwa et al. paper indicates that there was considerable variation in which lobes were present and examined in the NTP studies they reviewed, suggesting that some or most of study-to-study variation in the incidence of prostatic inflammation may be due to variations in the prostate lobes examined.

As pointed out in the EPA assessment document, suppurative inflammation and chronic (non-suppurative) inflammation can change into one another and it should also be noted that different pathologists may apply somewhat different criteria when diagnosing different types of inflammation. This is possibly one reason for why all types of inflammation were lumped together in the Suwa et al. (2001) paper. It is possible, but not clear from the Levine et al. report, that more than one pathologist was involved in reading the slides of their study, in which case diagnostic variation may have occurred. Of note, there is no mention of peer review of the histopathology of that study by Levine et al. and the prostate lesions were not subjected to a review by a pathology working group as was done for the liver lesions in the mouse study by Lish et al. (1984).

Thus, the incidence of prostatic inflammation (of any type) was increased only in the highest dose group. The incidence of prostatic inflammation in the control rats and those in the 0.3, 1.5, and 8.0 mg/kg-day groups in the Levine et al. (1983) study is similar, but low compared to the studies reviewed by Suwa et al. (2001); however, it is within the range expected to be found if one did not standardize which prostatic lobe was examined. This conclusion is at somewhat variance with the conclusion of the EPA assessment document that the control incidence was unusually low, which is based on only the data regarding suppurative inflammation in the control group. The EPA also considered only suppurative inflammation as major effect of RDX on the rat prostate, showing an increasing incidence with increasing dose at levels of 1.5 mg/kg-day and above. However, it appears that there is actually a shift from chronic to suppurative inflammation in the prostate with increasing RDX dose, which is statistically significant if one conducts a χ^2 -test with as categories

no lesions, chronic inflammation, and suppurative inflammation (please note that prostatic inflammation was scored by Levine et al. as either chronic inflammation or suppurative) across all treatment groups ($P < 0.0001$). The shift is almost complete in the 31 rats of the 40 mg/kg-day group as only two animals had (minimal) chronic inflammation and 18 had suppurative inflammation. Although this analysis should have taken into account mortality differences, the report of Levine et al. (1983) rat study does not contain data that would allow to do this, as pointed out in the EPA assessment document.

The considerable mortality differences between the experimental groups is a major factor in evaluating the results of the Levine et al. (1983) rat study. Although mean mortality was similar for the control and the 0.3, 1.5, and 8.0 mg/kg-day groups (mean survival between 21 and 22 months on study), significantly more rats died or were euthanized before the 24 month terminal end-point in the 1.5 and 8.0 mg/kg-day groups (27/52 [52%] and 26/55 [47%] rats, respectively) than in the control and 0.3 mg/kg-day groups (17/55 [(31%)] in both). In the highest dose group, there was considerable early mortality (mean survival was 14.6 months), and only 4/55 rats (7%) survived until the end in the 24 months study; 5 rats (9%) died before 6 months, 19 (35%) between 6 and 12 months, and 27 (49%) between 12 and 24 months. As pointed out above, the report of Levine et al. (1983) study does not contain data that allows a mortality-adjusted analysis. However, from the individual data presented in the Levine et al. report, one can deduct that in rats that died between 6 and 12 months, there was a 95% incidence of (sub)acute inflammation in highest dose prostates (mostly only mild-moderate), whereas in the other groups no inflammation or only a very low incidence of (sub)acute inflammation was found. After 12 months, many highest dose rats that died before the end of the study had suppurative inflammation (18 of 27; 67%), which did not occur in any rat of any treatment group before 12 months on study.

Papillary necrosis was found by Levine et al. (1983) in 15/19 (79%) highest dose rats that died between 6 and 12 months on study and in 18/31 (58%) highest dose rats that died between 12 and 24 months on study. In the highest dose group, hemorrhagic/suppurative cystitis was found in 17/19 (89%) rats that died between 6 and 12 months on study and in 18/31 (58%) rats that died between 12 and 24 months on study. The incidence of these lesions in lower dose groups and control rats was either very low or null; cystitis was found in 2 rats on the 0.3 mg/kg-day group and one rat in the 1.5 mg/kg-day group between 12 and 24 months on study, renal papillary necrosis was found in one rat in the 0.3 mg/kg-day group between 12 and 24 months on study. There was a high degree of correlation between the presence of the renal papillary necrosis and cystitis, as well as renal pericapsular inflammation/peritonitis on the one hand and suppurative prostate inflammation on the other in individual animals in the highest dose group. However, there was no such correlation between the renal effects and the cases of suppurative prostatic inflammation in the 1.5 and 8.0 mg/kg-day groups, with exception of one animal in the 0.3 mg/kg-day group while there was one animal in the 1.5 mg/kg-day group that had both prostatic suppurative inflammation and slight pyelonephritis. Thus, the increased number of rats dying before the end of the study in the 1.5 and 8.0 mg/kg-day groups could not have been due to such renal lesions. However, the early mortality in the highest dose group was no doubt driven by this renal toxicity, as pointed out in the EPA assessment document.

Significant early mortality only occurred in male rats at the highest dose level and the incidence of kidney and bladder changes, ranging from papillary necrosis to various types of inflammation, was also only increased in highest dose male rats. Therefore, it appears conceivable that the primary toxic effect of RDX was on the kidney with changes further down the urogenital tract (bladder and prostate) being secondary to the renal effects. The draft EPA assessment report comes to the same conclusion, but does not explain why the prostatic suppurative inflammation was somewhat increased in the 1.5 and 8.0 mg/kg-day groups in the absence of any marked renal toxicity at those dose levels. This would point to either a direct effect of RDX on the prostate or confounding factors, such as variability in the conduct of the histopathology, leading to an apparent increase in suppurative inflammation incidence in the 1.5 and 8.0 g/kg-day groups. Therefore, it seems not justified to use prostatic suppurative inflammation as "surrogate marker" for the renal and bladder effects of RDX as proposed by the EPA in its draft assessment document.

The mechanistic discussion in the EPA assessment document of the possibility that the renal/genitourinary effects of RDX might be mediated by GABA receptors is interesting and should be retained even though is highly speculative. The other hypothesis put forward by the EPA is that reduced urinary flow may have caused retrograde bacterial infection into the bladder and prostate and even the kidney. The EPA does not point out that this hypothesis is supported by the fact that the male animals in the 40 mg/kg-day group were fighting a lot and there is evidence in the literature that fighting may cause such urogenital infections in male rats (Creasy et al., 2012). Because of this fighting, all males in the highest dose group were individually housed from 30-40 weeks into the study, which introduced another factor different from the other treatment groups that may have affected the animals in the 40 mg/kg-day group in uncontrolled ways.

The marked gender difference in the renal toxicity due to RDX found for rats by Levine et al. (1983) is not discussed in the EPA document. However, there is precedent for a toxic chemical causing renal papillary necrosis selectively in male, not female, F344 rats (Neal et al., 2003) and several drugs are well known for gender-specificity in their ability to cause renal papillary necrosis (Bach and Nguyen, 1998; Brix, 2002).

References:

Neal GE, Judah DJ, Hard GG, Ito N. (2003) Differences in ethoxyquin nephrotoxicity between male and female F344 rats. *Food Chem Toxicol.* 41:193-200.

Creasy D, Bube A, de Rijk E, Kandori H, Kuwahara M, Masson R, Nolte T, Reams R, Regan K, Rehm S, Rogerson P, Whitney K. (2012) Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol Pathol.* 40(6 Suppl):40S-121S.

Bach PH, Nguyen TK. (1998) Renal papillary necrosis--40 years on. *Toxicol Pathol.* 26(1):73-91.

Brix AE. (2002) Renal papillary necrosis. *Toxicol Pathol.* 30(6):672-674.

3.b.ii. *Kidney and other urogenital system-specific toxicity values* (Section 2.1.1). *Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?*

Because of the comments made for section 3.b.i. *Kidney and other urogenital system hazard* (Sections 1.2.2, 1.3.1), the selection by the EPA of the prostatic suppurative inflammation for dose-response analysis appears not well justified. The use of the data regarding prostatic suppurative inflammation as a basis for a quantitative risk assessment by the EPA appears problematic because of the uncertainty of the influence of mortality differences between treatment groups, the absence of information about which prostate lobes were examined and whether this was standardized, the lack of information about diagnostic criteria and standardization of the histopathologic examination, and the lack of correlation between this prostatic lesion and renal toxicity in the 0.3 – 8.0 mg/kg-day groups. The prostatic effects appear mostly limited to the highest dose group (40 mg/kg-day), as are the renal effects of RDX exposure of male F344 rats.

3.b.v. *Kidney and other urogenital system-specific reference dose* (Section 2.1.4). *Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?*

Because of the comments for 3.b.ii. *Kidney and other urogenital system-specific toxicity values* (Section 2.1.1), it appears not really appropriate to calculate an oral reference dose for the kidney/urogenital effects as was done by the EPA. Omitting the reference dose for the kidney/urogenital effects of RDX would not reduce the overall toxicity evaluation because the overall reference dose is driven by the nervous system effects and not the kidney/urogenital effects.

3.e.i. *Cancer hazard* (Sections 1.2.5, 1.3.2). *There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusions.*

The hazard descriptor “suggestive evidence of carcinogenic potential” proposed by the EPA is supported by a well thought-out scientific rationale based on animal data only for liver tumors in female mice and, to a lesser extent, lung tumors in male and female mice and in the absence of human data and of convincingly supportive mechanistic data. The increase in liver tumors in male F344 rats at the highest RDX dose in the Levine et al. (1983) study is not truly convincing because of the lack of a dose-response for neoplastic nodules (= adenomas) and carcinomas combined. Thus, the scientific reasoning is sound that the induction of liver tumors by RDX in two species is weak and provides support for the hazard descriptor “suggestive evidence of carcinogenic potential” rather than the descriptor “likely to be carcinogenic to humans”. The scientific rationale for the conclusion that this descriptor applies to all routes of human exposure is reasonable.

3.e.ii. *Cancer-specific toxicity values* (Section 2.3.1). *As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, “When there is suggestive evidence, the Agency generally would not*

attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.” Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

The draft EPA assessment document adequately explains the rationale for quantitative analysis and justifies this analysis, taking into consideration the uncertainty in the data and the suggestive nature of the weight of evidence. The selection of the Lish et al. (1984) study for the purpose of quantitative analysis is scientifically supported and clearly described. In line 5 of page 1-26, a better term for “same cell line” would be “same cell type”.

4.c. Oral slope factor for cancer (Section 2.3.3–2.3.4). *The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?*

The justification for combining liver and lung tumors for the derivation of the oral slope factor is reasonable, but needs to be explicitly stated in the text and not only in Table 2-8.

5. Executive summary. *Does the executive summary clearly and adequately present the major conclusions of the assessment?*

The description of the urogenital effects in male rats should include specific mention of the renal effects, not only the prostatic effects. Because the observed suppurative prostatitis is part of a larger spectrum of prostatic inflammatory changes that are frequently found in aged F344 rats, the dose-response for this lesion found in male rats may in fact not reflect the overall incidence of all types of prostatitis combined in each dose group. The prostatic inflammation and renal/bladder effects may be inter-related, but this only occurred at the highest RDX dose tested. Therefore, it is not appropriate to calculate an oral reference dose for these kidney-urogenital effects and there seems no basis for the assertion that suppurative prostatitis is a “surrogate marker” for renal/bladder effects. Of note, this does not affect the overall oral reference dose, because that is based on the nervous system effects.

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Dr. Mary Boudreau

1) Literature search/study selection and evaluation.

The section on *Literature Search Strategy / Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Response: The literature search strategy | study selection and evaluation section in the IRIS assessment of RDX is clearly articulated. The online scientific databases and the Defense Technical Information Center (DTIC) database are clearly described, and the text is accompanied by tables (Table B-1 and B-2) of terms that were used to search the databases. The text is also accompanied by a flow chart (Figure LS-1) that summarizes the results of the search strategies and tables (Table LS-1 and LS-2) that outline the inclusion and exclusion criteria used to select literature that are considered pertinent to the purpose of the EPA review of RDX.

The section on the literature search and screening strategy is followed by a section on the selection of critical studies for the presentation of evidence and study evaluation. Both of these sections are well written and insightful, with tables that outline the studies under consideration and the rationale for the inclusion or exclusion of the study for the presentation of evidence in the IRIS assessment of RDX. The text describes the human and animal studies that were considered and recaps the findings on the experimental design of the study, how the test materials were characterized, the method used for the administration of test material, whether standardized procedures were used in the conduct of the study, the study endpoints and manner of evaluation, and how the data were recorded and reported.

The current structure of the *Literature Search Strategy / Study Selection and Evaluation* process is a rational approach that is clearly described. The sections within the text flow in a logical manner and the document demonstrates that EPA has gone to great lengths to specify the strengths and weaknesses of the data used for the assessment of RDX.

In terms of study evaluation, there is some concern in the interpretation of the data. On page xl of the draft review, for example, the text indicates that incomplete histopathological examinations were performed on rats and mice administered the mid-dose range of RDX in the 2-year bioassays conducted by Levine et al. (1983) and Lish et al. (1984). While this statement is technically correct, it is somewhat misleading to the reader. The study final report states that tissues from the controls and high dose animals were subjected to comprehensive histopathologic examinations, and all animals receiving mid-dose ranges of RDX were subjected to limited histopathologic examination of at least the following tissues: brain, gonads, heart, liver, lungs, kidneys, spleen, spinal cord, and masses. The review indicates that histopathological examination of a subset of tissues in mid-dose groups limited the ability to identify dose-related trends for tissues with incomplete histopathology. The review should clarify that all animals receiving mid-dose ranges of RDX were subjected to a comprehensive histopathologic examination of major

organs, including the brain, gonads, heart, liver, lungs, kidneys, spleen, spinal cord, and masses.

I am not aware of any additional peer-reviewed studies from the primary scientific literature that should be considered in the toxicological assessment of RDX.

- 2) **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans ([Sweeney et al., 2012a](#); [Sweeney et al., 2012b](#)).

- 2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

Response: The revised PBPK model appears to adequately represent the RDX toxicokinetics based on available published studies in rats and the one study in mice. The model was fit to several published data sets following oral exposure to RDX dissolved in water, in dry capsules, and as coarse-grains. The model was then calibrated against an additional two additional sets of time course data, and the overall predictions were within a factor of 1.5 of the measured values. The model assumptions, in particular 100% absorption and 100% metabolism for RDX, were clearly stated. The absorption value appears to vary greatly in the published literature - values in animals of approximately 50 – 90%. These assumptions affected the metabolic rate constant. In the discussion on the sensitivity tests conducted on the model, this was acknowledged as an uncertainty that appeared to be dependent upon the form of RDX.

- 2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

Response: Studies in animals have provided enough information to establish approximate blood levels of RDX that are associated with certain toxic responses, even though this data is lacking in humans. The EPA's revised model allows for good extrapolation of the results of these animal studies to humans. Of concern is the variability in RDX elimination data for humans, with $t_{1/2}$ for elimination ranging from 15 – 29 h, and the significantly lower $t_{1/2}$ for elimination of approximately 7 h in rats after oral dosing, as reported by Sweeney.

- 2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

Response: Composite uncertainty factors selected by Sweeney et al. (2012) included a UF_A set to 3 for interspecies variation and a DAF value set to 10 was used because the individual human toxicokinetic profiles provided insufficient information for confident assessment of the interindividual toxicokinetic differences. Due to the

limited availability of data from human exposure to RDX, the uncertainty factor of 10 is quite appropriate.

- 3) **Hazard identification and dose-response assessment.** In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system- specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) to reach the following conclusions.

[Note: As suggested by the Chemical Assessment Advisory Committee panel that reviewed the draft IRIS assessment of benzo[a]pyrene, the charge questions in this section are organized by health outcome, with a question on each hazard identification followed by questions on the corresponding organ/system-specific toxicity values. This suggestion, however, entails some redundancy, as some questions apply equally to multiple health outcomes.]

3a) **Nervous system effects**

3b) **Kidney and other urogenital system effects**

3c) **Developmental and reproductive system effects**

3d) **Other noncancerous hazards**

3e) **Cancer.**

- i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

Response: A presentation and synthesis of the evidence for RDX carcinogenesis is provided in Chapter 1, *Hazard Identification*, Section 1.2.5 and 1.3.2 of the IRIS toxicological review of RDX. The carcinogenicity of RDX was assessed in 2-year bioassays in mice by Lish et al (1984) and in rats by Levine et al. (1983). Both of these studies were conducted at the same facility, under the direction of the same laboratory, and used the same source of well-characterized RDX materials. The rationale for conducting these studies was to provide data on the mammalian toxicology of RDX, as part of a comprehensive study to establish a water quality criterion for RDX and other compounds unique to the munitions industry.

RDX has low solubility in water (59.7 mg/L), but the organic carbon/water partition coefficient values indicates a potential for RDX mobilize in soil, and the vapor pressure (4.1×10^{-9} mm Hg at 20 °C) suggests that RDX likely exists as particulate matter in the air. RDX may be adsorbed onto the surfaces of soils and may be absorbed by plants via ground water contamination.

Absorption following oral exposure has been demonstrated in humans and laboratory animals. Quantitative estimates of oral absorption are not available in humans, although crude estimates were obtained via accidental exposures of adults and one child. Estimates in animals indicate that approximately 50 – 90% of RDX is bioavailable following oral exposure, with purity and particle size parameters

influencing bioavailability. Studies to evaluate the exposure to RDX via inhalation exposure were not found; however, as particulate matter in the air, inhalation represents a potential route of exposure. *In vitro* studies have examined the percutaneous absorption of RDX from solvents and low- and high-carbon soils. The results of this work showed that the medium influenced absorption, with as much as 5 – 6% of applied RDX absorbed through excised human and pig skin. Exposure to RDX in the general population is confined primarily to individuals living around active or formally-active military facilities; however, oral, inhalation, and dermal routes of exposure to RDX are relevant in the assessment of human health risks from RDX exposure and in the application of all routes for the potential of cancer risks. In *Guidelines for Carcinogen Risk Assessment*, when tumors occur at a site other than the point of initial contact, the descriptor generally applies to all exposure routes that have not been adequately tested at sufficient doses. The observations of tumors in the lungs and livers of the B6C3F1 mouse confirm that the correct descriptor was selected.

There are concerns, however, regarding the two bioassays that were used for cancer determinations in the review of RDX. Both studies suffered from high mortality rates among animals in the high dose groups. In the Levine et al. (1983) study, 75 F344 rats per sex per treatment were loaded on the study. The experimental design of the study planned for 10 male and 10 females to be used for interim sacrifices at 6 and 12 months, with 55 rats per sex per treatment allocated for the 24-month scheduled sacrifice. At termination, only 4/55 males and 28/55 females in the 40 mg/kg diet survived to scheduled sacrifice. The Lish et al (1984) study in male and female B6C3F1 mice was designed in a similar manner as the Levine et al. (1983) study in rats. In the Lish et al. study (1984), 85 mice per sex per dose were loaded on the study. The experimental design planned for interim sacrifices of 10 male and 10 female mice at 6 and 12 months. Mortality was high early in the study, so the investigators amended the protocol and lowered the RDX high dose 175 to 100 mg/kg diet. At termination, 27/65 males and 31/65 females in the high dose (175/100 mg/kg diet) group survived to scheduled sacrifice. Due to the high mortality rates ($\geq 50\%$) among animals in the highest dose groups, the data for these animals should be excluded from consideration, as inclusion would tend to exaggerate the weight of evidence.

Another concern with both the Levine et al. (1983) and Lish et al (1984) studies is that an independent quality assessment laboratory was not used to conduct an external pathology data review and quality assessment. In view of the low incidence of liver tumors in female mice (B6C3F1 mice have a propensity for liver tumors), a quality assessment of the Pathology calls, especially adenomas and carcinomas, would seem warranted. Differences in diagnostic criteria may influence reported tumor rates. For example, the NTP uses rigorous histopathology quality assurance methods and peer review procedures to ensure consistency and comparability of tumor diagnosis from study to study. Biological factors, such as body weight, may also affect tumor incidence; increased body weights are associated with increased incidences of liver tumors in B6C3F1 mice. Body weight increases was not a factor in the Lish et al. (1984) study in mice, as mean male body weights at week 104 were ~ 36 – 38 g and mean female body weights were ~ 15 – 22 g; mean body weights of NTP B6C3F1

historical controls at termination from 11 chronic studies were 50 g for male and ~ 58 g for female mice. The rodent diet used for the rats and mice in these two studies was rodent chow 5002, which has a relatively low fat content of 5 – 6.3%.

The disparity of hepatocellular neoplasms in the control mice; a very low (1.5%) incidence of neoplasms in the female control group compared to the relatively high incidence (31.7%) of neoplasms in the male control group was a concern with the Lish et al. (1984) study and reduced the confidence in accuracy of these data. Mice, more so than rats, are susceptible to spontaneous liver tumors, and B6C3F1 mice manifest a high rate of spontaneous liver tumors, especially in males. Historical control incidences for the combination of hepatocellular adenoma and carcinoma in NTP B6C3F1 mice as of August 2016 were 60 – 84% (mean 71.09%) from all routes of administration in males and 16 – 73% (mean 34.42%) from all routes in females and was based on 550 or 549 mice, respectively

(<http://ntp.niehs.nih.gov/results/dbsearch/historical/>). Incidences of hepatocellular neoplasms were significantly lower in the female control group than historical controls. The combination of hepatocellular adenomas and carcinomas, though not statistically significant, was 42.4% in the 35 mg RDX/kg diet dose group of male mice; however, the combined incidence of 31.7% in control males resulted in equivocal evidence by RDX. Although the concurrent control group is always the most appropriate control for decision making, there are instances, such as when a tumor shows a borderline increase relative to concurrent controls, when historical controls can aid in the overall evaluation of tumor incidence data. In the Lish et al. (1984) study, the call would have been “equivocal” for both male and female mice.

In 2005, the EPA classified RDX as a potential carcinogen, and the change in the classification along with evidence of RDX contamination of ground water near military munition installations prompted a reevaluation of cancer data by an independent Pathology Working Group (PWG) of tissues (female only) from the Lish et al. study (1984). In the reevaluation of hepatocellular neoplasms in female mice, the PWG found many of the hepatocellular adenomas did not meet the diagnostic criteria and were reclassified as foci of cytoplasmic alteration. As a result of this action, only the 35 mg RDX/kg diet group showed a significant increase (15.6%) when compared to the study control group (1.5%). Furthermore, the PWG was limited in its diagnosis; necropsy and histopathological records were not made available, fixed tissue remnants were not available from all animals, and irregular technical aspects were noted in the size and number of liver sections prepared for the individual animals, with notation to indicate some mice with only one liver section when the minimum is two. An obvious shortcoming to the PWG reevaluation is that they did not reevaluate tissue sections from male mice. In general, a PWG evaluation usually includes sections from both sexes and from all dose groups. The likelihood exists that if the diagnostic criteria changed the classification of neoplasms in female mice then it should have a similar effect on the diagnosis of neoplasms in male mice. A dose-related increased trend was observed in female mice, when the lung data were analyzed using a one-sided Cochran-Armitage trend test by the EPA. The incidences of hepatocellular adenomas and carcinomas were very low in the rat study and RDX treated groups were not different from controls in either sex.

Alveolar/bronchiolar neoplasms were observed in male and female mice in the Lish et al. (1984) study. Rats were not sensitive to the development of neoplasms at this site. There is concern with the data from this site as well, although the control groups are similar and the number of neoplasms in the control animals was similar to that reported for historical controls. Due to the high mortality in the 175/100 mg RDX/kg diet, the presence of neoplasms in this group should be discounted. The Cochran-Armitage trend test was used to determine a dose-related effect. In the same NTP cohort of B6C3F1 mice that was cited for liver tumors, the incidence for alveolar/bronchiolar carcinoma or adenoma in historical controls range from 16 – 38% (mean 26%) in males and 2 – 18% (mean 10.21%) in females. A dose-related increased trend was observed in female mice, when the lung data were analyzed using a one-sided Cochran-Armitage test by the EPA.

In *Guidelines for Carcinogen Risk Assessment*, studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits. This was a difficult task given the low number of hepatocellular neoplasms in female control mice, the high number of neoplasms in the male control mice, and a general lack of concordance between mice and rats. The descriptor, “Suggestive evidence of carcinogenic potential”, is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged insufficient for a stronger conclusion. This appears to be the case for RDX; the evidence of carcinogenic potential in the liver and lungs/bronchi is weak; however, the number of neoplasms in both male and female mice raises the concern for potential human carcinogenic risks.

- ii) **Cancer-specific toxicity values** (Section 2.3.1). As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, “When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.” Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the [Lish et al. \(1984\)](#) study for this purpose scientifically supported and clearly described?

Response: A presentation of the evidence for a quantitative analysis of RDX carcinogenesis is provided in Chapter 2, *Dose-Response Analysis*, and Section 2.3.1 of the Toxicological Review of RDX. A dose-response assessment estimates the potential risks to humans at the exposure levels of interest and should consider the quality of the available data. The draft assessment clearly indicates that the large number of animals tested at multiple dose levels in the two-year bioassays studies by Levine et al., 1983 in F344 rats and Lish et al., 1984 in B6C3F1 mice were the impetus for considering the cancer response for dose-response analysis. As mentioned previously, the interpretation of the data for the carcinogenicity of RDX is not straight forward. The scientific support for the selection of the cancer descriptor for RDX is only suggestive evidence from the two bioassays that demonstrated neoplasms in animals. Although these studies were well conducted, there were a number of issues in the conduct of the histopathology evaluations, in particular with regards to the hepatocellular neoplasms in the control mice of both sexes.

The study by Lish et al. (1984) formed the primary tumor data for the quantitative dose response analysis. The liver tumor data from the Levine study (1983) and low lung tumors in both sexes of rats were less robust than the data from female mice in the Lish et al. study, which also was weak and lacked confidence due to issues mentioned above. The cancer endpoints used for the dose response analysis were the combined adenomas and carcinomas for hepatocellular neoplasms and for alveolar/bronchiolar neoplasms in female mice. Section 2.3.1 of the review clearly states the usefulness of the analysis in providing a sense of magnitude of the potential for carcinogenesis, and the use of the reevaluated female liver data by the PWG (Parker et al. 2006) along with the female lung tumor data provides greater scientific support for the analysis. This is clearly described in the draft assessment.

- 4) **Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) in the following analyses.

4a) **Oral reference dose for effects other than cancer**

4b) **Inhalation reference concentration for effects other than cancer**

4c) **Oral slope factor for cancer (Section 2.3.3–2.3.4).** The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

Response: An oral slope factor (OSF) is used to estimate the risk of cancer associated with the oral exposure to a potentially carcinogenic agent; it is a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to an agent for a lifetime of 70 years. For RDX, the incidences of both liver and lung combined neoplasms (adenomas and carcinomas) in female mice were used to derive the RDX OSF for cancer.

The analysis of cancer data is scientifically supported when the evidence includes a well-conducted study. The review clearly describes which studies are given the greatest weight and the reasons for their selection, as well as their reason for rejection. The Lish et al. (1984) study was conducted in accordance with the FDA guidelines, *Good Laboratory Practices for Non-Clinical Laboratory Studies*, and in compliance with a study protocol and its amendments that were approved by an institutional animal care and use committee. This study was used for the derivation of the oral slope factor.

Section 2.3.3 clearly articulates the steps and calculations used to determine the oral slope factor and describes its limitations; while Table 2.7 shows the result of each calculated factor that was used to determine the oral slope factor for each tumor type separately and in combination.

Section 2.3.4 discusses and summarizes the uncertainties that were considered in deriving the oral slope factor for cancer induced by RDX and what potential impact the uncertainty might have on the cancer value. At the heart of the cancer guidelines for risk assessment is a critical assessment of the available literature and selection of the appropriate studies to provide accurate data for cancer risk. Section 2.3.4 outlines in tabular form (Table 2.8) the decision and justification for the selection of the Lish et al. (1984) study as the basis for the cancer data, with the female mice being the most sensitive species and sex. The review describes the uncertainties considered in determining the cancer value for RDX, weighs

the potential impact of the uncertainty on the cancer value, states the decision reached in regards to the uncertainty, and justifies how and why the decision was reached.

- 5) **Executive summary.** Does the executive summary clearly and adequately present the major conclusions of the assessment?

Response: The Executive Summary does summarize the major conclusions pertaining to hazard identification and the dose-response analysis. On page xxiii of the Executive Summary, a paragraph break is needed after the sentence, "There is no known MOA for male reproductive effects of RDX exposure." The next sentence does not relate to the male reproductive effects but speaks to the evidence for effects in other organs/systems.

On page xxv, the paragraphs titled, *Effects other than cancer observed following inhalation exposure* and *Inhalation reference concentration (RfC) for effects other than cancer*, should be combined. There is no available literature to support the identification of hazards following inhalation and a reference concentration cannot be determined. This should be stated simply in a single paragraph.

On page xxvi, wouldn't it be more correct to record the oral slope factor (OSF) as 4×10^{-2} (mg/kg-day)⁻¹, rather than 0.04 per mg/kg-day?

The remainder of the Executive Summary was concise, well written, and described completely the findings of the draft assessment.

Dr. James Bruckner

2.a. The conclusions reached by the EPA following its evaluation of the PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b) are well supported scientifically. EPA staff did a thorough and accurate job reviewing and summarizing much of what is known about the oral absorption of different forms/preparations of RDX, as well as the compound's distribution, metabolism and excretion. More attention, however, should be devoted in Section C.1.1. to supplement information about the distribution of RDX to the brain and other target tissues. It is noteworthy that RDX concentrations in the serum and cerebrospinal fluid of the pediatric subject of Woody et al. (1986) were quite similar. RDX levels in whole brain and plasma of minipigs were reported by Schneider et al. (1977) to be 7.0 and 4.7 µg/g, respectively. The brain:plasma ratio in rats 4 to 24 hours after ingestion of 100 mg/kg of RDX was relatively constant. Bannon et al. (2009) observed similar RDX time profiles for brain and blood in rats, though the compound appeared to be cleared somewhat more rapidly from the bloodstream. Although the EPA chose not to use PBPK-simulated brain RDX concentrations as a dosimeter for neurotoxicity risk assessment, the aforementioned experimental findings lend support to the decision to use plasma as a surrogate.

There are some published data on distribution of RDX to other potential target tissues. Schneider et al. (1977) found higher RDX concentrations in brain and kidney than other tissues that were monitored in rats and minipigs. Concentrations of the parent compound in the liver were relatively low, possibly due to extensive metabolism there. Sweeney et al. (2012b) concluded that liver-generated RDX metabolites were the most likely causative agents associated with one of their proposed modes of action.

There is no mention in Section C.1.1. of deposition of RDX in adipose tissue. The concentration of RDX measured by Schneider et al. (1977) in fat of minipigs 24 hours after oral administration of 100 mg/kg was comparable to those in brain and kidney. This was also the case with rats after consumption of RDX-saturated water for 90 days (Schneider et al., 1978). RDX clearly does not accumulate in fat or in other tissues. RDX levels in blood/plasma versus fat are key to derivation of accurate partition coefficients (PFs). Optimized/fitted PFs (5.57 and 7.55) used in PBPK modeling are higher than would be anticipated from these limited empirical data. It is noted on page C-15 that upon further development of the Sweeney et al. (2012a) rat model by the EPA, fat and slowly perfused partition coefficients (PF and PS) were set to values calculated by Krishnan et al. (2009), resulting in slightly worse fits of simulated and experimental time-course data. Krishnan and coworkers' technique for calculation of partition coefficients for organic chemicals takes into account tissues' neutral lipid, phospholipid and water content. These calculations do not take cellular or plasma protein binding into account. Protein binding of RDX may be substantial.

The PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b), as well as the revised EPA model, adequately represent RDX toxicokinetics (TK) for oral exposure. It is nice to see reasonable agreement of simulated RDX time-courses with the rat, mouse and human empirical data that are available. Concurrence might be anticipated, however, for models for which key input parameters (i.e., absorption and metabolic rate constants) are derived by data

fitting. The success of simulating TK data from experiments employing different forms/preparations of RDX is noteworthy.

My primary concern about modeling the TK of RDX is the early time-period (i.e., initial hours) following RDX ingestion. The PBPK models are very sensitive to oral bioavailability and to metabolic rate constant. It is reasonable to assume 100% oral bioavailability, in light of experiments (e.g., Schneider et al., 1977) showing up to 90% recovery of a 50 mg/kg oral dose of ¹⁴C-RDX within 24 hours. Absorption rate constants are necessarily optimized to fit measured blood concentration data. I am concerned that the available data sets lack sampling time-points during the uptake phase. In a number of instances blood levels appear at or near maximal at the first time-point, though the peak level may have been missed. Accurate absorption rate constants cannot be calculated from published TK data. C_{max} is underpredicted and overpredicted in several instances in Figures C-3, C-4, C-6 and C-7. The importance of this is diminished by the decision to employ AUC as the dosimeter for neurotoxicity, although inaccurate C_{max} values detract from the accuracy of AUCs.

Overall, the document's authors have done an excellent job writing Appendix C. The model assumptions and parameter discussion and selections are clearly presented and supported scientifically. The discussion of model uncertainties and limitations is accurate and succinct. The reasoning for selection of the rat over the mouse model is sound.

2.b. The reasoning for selection of AUC rather than peak concentration (C_{max}) as the dose metric for neurotoxicity is clearly explained and quite logical. As I described above, limited empirical TK data show concordance between blood and brain RDX levels over time following exposure. It should also be pointed out in the text on page 2-8 that AUC is a better representation of the adverse effect of interest than is RDX concentration at a single point in time. Seizures in humans and rodents typically last for a period of hours to a day or more, dependent upon treatment interventions. Published 24-hour time-courses of blood and brain RDX levels (e.g., Bannon et al., 2009) appear to coincide with symptomatology. It may be reasonable to assume that seizures or hyperreactivity would be manifest as long as a threshold blood/brain concentration of RDX (e.g., 8 µg/g by Williams et al., 2011) was present.

The decision not to use the mouse PBPK model for noncancer endpoints is clearly explained on pages C30 and C31. The uncertainties are numerous and logical.

2.c. It is standard practice to adopt an interspecies uncertainty factor of 10 to account for potential differences in TK and toxicodynamics in the absence of information about variability within human populations. There is a paucity of information on intersubject variability of RDX TK or toxicity. PBPK modeling described in Appendix C identifies absorption and metabolic rate constants as two key input parameters that substantially influence model predictions. The rate of metabolic clearance could vary significantly among humans, but no human data on RDX metabolism are available.

3.d. Human and animal studies that are available support the decision not to draw any conclusions as to whether ocular, musculoskeletal, cardiovascular, immune or gastrointestinal (GI) effects are human hazards of RDX exposure. In most instances there is little or no evidence

that RDX directly affects several of these organ systems. Were the muscular twitching and myalgia experienced by severely poisoned patients of Stone et al. (1969) due to musculoskeletal or CNS effects of RDX? Stone et al. saw a large increase in SGOT activity, which they believed may have been due to muscle damage. Similarly, was sinus tachycardia an indirect consequence of systemic toxicity or due to a direct effect on the heart? GI upset, however, does appear to be due to direct irritation of the GI mucosa by RDX. Nausea and vomiting are frequent clinical manifestations of ingestion of large amounts of RDX. Kuchkardali et al. (2003) observed erosive gastroduodenitis by endoscopy in 3 of 5 RDX poisoning victims who consumed neurotoxic quantities of the compound.

There have been a number of reports of liver effects of RDX in laboratory animals and humans. Such effects, when observed, are usually quite mild and transient. Levine et al. (1977, 1978) reported modest increases in liver weight in rats given a single oral dose of 100 mg RDX/kg, or in rats consuming ≥ 100 mg RDX/kg daily for 13 weeks in their feed. No histopathological changes or increases in serum enzyme activities accompanied the modest hepatomegaly. French et al. (1976), in an Abstract, described proliferation of the hepatic smooth endoplasmic reticulum (SER) in rats 24, 48 and 120 hours after rats were gavaged with 100 mg RDX/kg. SER proliferation, of course, is indicative of induction of cytochrome P450s (CYPs). Xenobiotics frequently induce the CYPs that metabolize them. Bhushan et al. (2003) demonstrated that CYP2B4 from rabbit liver catalyzed reduction of RDX, causing double denitration, which led to hydrolytic ring cleavage. Apparently there are few other published data on mammalian metabolism of RDX, other than a report by Major et al. (2007) confirming formation of ring cleavage products and possible nitroso metabolites in minipigs.

There have been occasional reports of slight, transient increases in serum enzyme levels, indicative of very modest hepatocytotoxicity in humans who experienced RDX-induced seizures. In other cases there were no alterations of indices of liver injury. Kucukardali et al. (2003) did measure elevations in several serum enzymes in 4 of 5 patients the third day after experiencing seizures. The enzyme levels progressively returned to normal by day 10. These individuals also exhibited elevation of creatine phosphokinase (CPK), which may be indicative of skeletal or smooth muscular injury. Stone et al. (1969) observed a marked, but transient increase in SGOT activity, which they tentatively attributed to skeletal muscular injury. Testud et al. (2006) found elevated CPK and myoglobin levels in an Octogen-poisoning victim. The clinicians attributed these findings to muscle damage secondary to seizures.

It may be worthwhile to add a description of animal and human study findings on the potential hepatotoxicity of RDX to Subsection 1.3.1. Slightly elevated serum transaminases and hepatomegaly have been seen in dogs poisoned by RDX (Fishkin et al., 2008; de Kramer et al., 1992). There are rat dose-response data for single and repeated oral exposures. There are a number of case reports of mild hepatocytotoxicity in persons who ingested high doses of RDX, although the dosage-levels are uncertain. It is noteworthy that manifestations (clinical chemistry indices and/or histopathology) of kidney injury frequently accompanied alterations of liver parameters.

4.b. I agree data are insufficient to derive an inhalation reference concentration for RDX.

Dr. George Cobb

GENERAL:

The Agency and authors of this IRIS document have done a good job with much incomplete data that needs to be augmented to reduce the high uncertainties about the adverse health effects of RDX.

What are new data that has become available and how were they used to reduce cancer risk to suggestive from probable??

It seems that the only data considered in Parker et al 2006 are from Lish et al 1984 or military reevaluation of these data.

Evaluate Pan results and Smith results for RDX and convulsions to address the text indicating that no neurotox results were observed until convulsion was seen.

Section 1.1.2 Absorption of RDX by brain and other tissues is also presented in Pan et al. Pan et al measured organ weights following chronic dose.

Reliance on conformity to monotonic dose response models as a reason for inclusion/exclusion of effect in a situation with this little data is erroneous and should not be allowed. If this was a compound with an incomplete biotransformation pathway and an uncertain spectrum of modes of action. If MOA, biotransformation and the molecular activations were clearly understood, the shape of dose responses could be compared to expectations, but with the dearth of data, assumptions of monotonic responses can not be made.

Similarly, assumptions that males and females should respond similarly to RDX exposure or that dose responses is not supported. We only need to consider that VIOXX was removed from the market for causing male hypertension to find a clear example of starkly different responses from males and females.

Q1) It would also seem that the data on toxicity of MNX, and TNX should be included or considered here especially given their

Reductive transformation products to N-nitroso compounds are:

- 1) produced in the GI tract
- 2) present in the blood of dosed mammals
- 3) present in groundwaters near some munitions facilities
- 4) and structurally similar to di-N-nitroso-piperazines, which are used as positive controls in inhalational cancer studies.

The known production of these transformation products and their mutagenicity support position of likely carcinogen. The literature included in this assessment should be expanded to include these terms.

Please note that in HERO Net the Guo, L; Xu, H; Chen, Y; Chang, Y 1985 reference has no journal listing and is thus inaccessible through the database.

Metabolite profiling of [C-14]hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. Major, MA et al.

Swine study Only performed single dose, but did get limited toxicokinetic data. Data/report should be evaluated in context of Pan et al RDX dosing.

RDX transfer to brain also noted in Pan et al. and should be included in Appendices on p.B-6 especially given that the citation is mentioned later in the appendix. There is inferential evidence that reductive transformation is occurring after RDX in transported from the GI tract. She also found that brain and total body weights were not different after 28 day exposures of B6C3F1 mice to doses up to and including 500 mg/kg, but that liver and kidney weights increased.

Smith et al., two year dosing with TNX caused significant reproductive effects and microRNA alterations. This should be noted in the risk assessment as possible adverse effect from long term exposure to RDX or to groundwater containing MNX.

Granted many of the studies noted above may have been performed without all needed criteria set forth for inclusion in this assessment. With lack of support for these evaluations we are in the unfortunate situation of basing national decisions on data that are 30 years old, much of which is questionable, as indicated by the revision of histopathology interpretation and comparing results to controls other than the study controls. EPA is left with a series of incomplete studies from which it is difficult if not impossible to reasonably draw conclusions. One or two well designed follow-up studies to the Lish 1984 effort could have and should have filled these data gaps over a decade ago. Manufacturers or users must provide adequate data to allow reliable assessments of toxicity (xxvii L10).

It would seem that if the Lish and other DOD technical reports are considered, several peer reviewed publications should also be included.

Carcinogenesis (1.2.5):

Approach to reevaluate data from study with dosing and other flaws is inappropriate. We are beginning to see a troubling pattern in EPA programs where lack of data is forcing agencies to reevaluate marginal data instead of having producers or users generate data that are of sufficient quality to use in hazard and risk assessments. If we are to accept that "Because the PWG analysis reflects more recent histopathological criteria for the grading of tumors, the incidence of hepatocellular adenomas or carcinomas as reported by Parker et al. (2006) were considered the more reliable measure of liver tumor response in female mice from the Lish et al. (1984) bioassay." We should also expect that MORE RELIABLE study designs be incorporated into a repeat of this study in an EPA or NTP lab. And this should be done before any diminution of toxicity characteristics in the IRIS program.

Does the statement "In male mice from the Lish et al. (1984) study, the incidences of hepatocellular carcinomas 22 in treated groups were higher than in the control, and the combined incidences of hepatocellular 23 adenomas or carcinomas of male mice were higher in three of four treated groups than in the 24 control; however, there were no statistically significant trends in either case." Mean that the dose response was non-monotonic or that differences between control and each treatment was not significant??

Similar Question of interpretation of Hart et al (1976) data. Since there data were only collected at the highest dose, how can the dose dependent statement be made??

P.1-62; See footnote, especially last sentence: Overall does comparison to NTP historical control result in data showing increased or decreased risk?? Does using NTP data introduce an uncertainty that is not included in the uncertainty factors that are included in the assessment provided?? Why was NTP control data used instead of study controls?? Did more advanced NTP methods find any increases in tumor incidences in controls, which would place control data more in line with historical trends??

If significant difference in lung tumors was seen why lower cancer risk designation??

Mechanistic Evidence (1-68): Little mechanistic evidence is direct result limited funding from federal sources. This should be focus in the future.

P. 1-68, LL38: What is the basis for the inclusion of the word aberrant?? Seems biased.

Limited MNX or TNX in swine urine should not be used to diminish role of MNX or TNX in mutation or carcinogenesis. Both compounds are found in brain, liver and kidney during mouse feeding studies. MicroRNA alterations in mice have been observed, and microsatellite alterations have been observed in multigenerational aqueous TNX exposures.

Sweeney (2012) reference is a review of data and does not provide any empirical data to body or evidence regarding RDX toxicity, including carcinogenesis. The assertion that there are limited evidence supporting N-nitroso uptake into tissues is erroneous. Studies that have sought to quantify these metabolites with methods sufficiently sensitive to determine them have found them. The majority of studies have not attempted to quantify N-nitroso compounds as they are difficult to determine, requiring rapid preparation and analysis.

P. 1-69; LL23-26: The conclusion is not supported by statement regarding MNX and TNX determination in tissues in the next sentence.

"In summary, the available evidence indicates that RDX is likely not mutagenic (see Appendix C, Section C.3.2), although anaerobically-derived N-nitroso metabolites have demonstrated some genotoxic potential. While these metabolites have been measured in the mouse (Pan et al., 2007b) and minipig (Musick et al., 2010; Major et al., 2007), they have not been identified in humans, and may not be the predominant metabolites of RDX." The simplest evidence of this error is the fact that oxons of organophosphorous insecticides are not the primary metabolites of that class of pesticide, but they are the causative agents in OP poisoning.

Further the lack of toxicokinetic data in rodents and swine is a direct result of lack of funds to appropriately conduct follow-up studies. In cases such as this, claims that data are not available should never be used. It does stand to reason that the MOA is not well understood (which also refutes the need for the dose response to be monotonic as noted in comments above), but there is evidence that RDX related N-nitroso compounds are found in mammalian tissues following dosing and that N-nitroso compounds are mutagenic. Finally there similarity in structure of di-nitropipirazine and RDX adds further indication that lung and nasopharyngeal tumors are caused by cyclic aliphatic N-nitro compounds and their N-nitroso transformation products.

What is really needed is a well-designed study performed by EPA or NIH to address these questions not rehashes of old studies in attempts to decrease toxicity profiles of RDX and other constituents in the registry.

P.1-74: In my view it is inappropriate to state that “the evidence of carcinogenicity outside the B6C3F1 mouse is not robust, and this factor was decisive in choosing a hazard descriptor” If the data from the primary producer/end user are insufficiently robust, the hazard identification should be more conservative (ie indicative of higher risk) until robust data are provided.

Added Questions

In addition to Zhang and Pan microRNA data, Smith et al demonstrated microsatellite alterations in mice exposed to TNX over multiple generations.

Studies in mammalian systems also demonstrate the reduction of nitro groups to from N-nitroso compounds. There is no indication of the affects that disease state or antibiotics have on the GI tract microbiome and other GI tract characteristics that may influence the reductive transformation of RXD. This should be explicitly stated in the Summary of the IRIS document (P 1-2) as well as in the body of the text. Omission of this information is a serious omission in the risk assessment as exposures are unlikely to be IP or IV and much of the toxicokinetic data use IV/IP routes of administration thus missing the mutagenic reductive transformation products.

Data from factory workers did find cognitive impairments, but these were discounted due to lack of control for alcohol consumption. Co-exposure exclusion rationales are weak as any chemical manufacturing facility will have co-exposures and any life style that includes manufacturing will present exposures to hazardous chemicals (eg air pollutants, pesticides, plasticizers, flame retardants, etc). Also, to what extent would alcohol consumption need have differed in these cohorts to see these effects in the absence of RDX consumption and what is the likelihood of such differentials in control and treatment groups. What is the evidence for TNT neurodegenerative effects?? Further what is the likelihood that humans (military personnel) exposed to RDX would not consume alcohol?? What is the rationale for no data having been generate by US since the last report in 2005?? All of this points to the need to include endpoints much more refined than seizures as neurologic endpoints.

Figure 24 of MacPhail et al shows declines in motor activities following low doses of RDX. The nature of the graphs and the lack of tabulated data with summary statistics makes it impossible to

truly assess if there are non-monotonic responses or lack of responses. Similarly figure 31 shows what appears to be significantly different responses to acoustic stimuli in treatment groups at all doses (as low as 1 mg/kg). These data seem to be more sensitive than are responses shown in Tables D-7 through D15 in Appendices. Thus seizures are unlikely an appropriate endpoint for neurotoxic effects.

USEPA workshop proceedings edited by Gerity and Henry 1990 offer possible approaches to estimate inhalation dose from dermal absorption data. Additionally data from di-N-nitropiperazines coupled with lung absorption modeling may offer a reasonable approach to assessing bioaccumulation via the lung and depending on the PBPK data availability for nitro piperazines a possible tie to the current PBPK modeling for RDX.

Smith et al should be consulted for supplemental data regarding developmental toxicity of TNX. TNX caused decreased weight and decreased survival in treatment groups receiving 10 and 100 ug/L, with effect sizes as large as 20%.

USEPA 2012d. What is the basis for limited/no surface runoff??

Dr. David Eastmond

- **1. Literature search/study selection and evaluation.** The section on Literature Search Strategy | Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The search strategy and study selection criteria seem reasonable and are adequately described. I do wonder if studies should be excluded simply because the information in the abstract is considered to be inadequate. While this is a practical approach, it is possible that studies with valuable information could be excluded using this criterion. I am unaware of additional studies on RDX that have not been included.

- 3e. (i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

I believe that there are plausible scientific arguments for four different possible listings for RDX. These include "Likely to Be Carcinogenic to Humans," "Suggestive Evidence of Carcinogenic Potential," "Inadequate Information to Assess Carcinogenic Potential," and "Not Likely to Be Carcinogenic to Humans." The choice depends largely upon how one chooses to interpret the animal cancer bioassay results and their significance. In this case, I believe that the EPA has made the best decision in concluding that there is "suggestive evidence of carcinogenic potential". The key considerations for this recommendation are outlined below.

Liver tumor data:

- . Male mice: Although there are suggestive increases in liver tumors seen, none of the increases appear to be statistically significant using either a trend test nor by pairwise comparisons. The incidences of these types of tumor are quite variable in mice and the observed increases fall within the range seen historically in controls (Haseman et al., 1985). The high dose incidence of combined tumors falls near the high end of the historical range.
- .
- . Female mice: Increasing and statistically significant increases in liver tumors were seen. However, there are multiple reasons to use caution in interpreting the conclusions. These include:
 - . There was very high mortality at the 175/100 mg/kg dose in the female (and male) mice. At least during the period when the 175 mg/kg dose was administered, this appears to have exceeded the maximum tolerated dose and the panel should discuss

whether this dose should be eliminated from the analyses for both the liver and the lung tumors for both male and female mice.

- . Unusually low incidence of liver tumors in the control female mice. In Lish et al. (1984) it indicates that the control incidence is significantly lower than that of the historical controls from the test lab and lower than that seen by the NTP. If a typical incidence of 8% seen by the NTP for this strain had occurred, the observed trend would be weakened substantially and it is unlikely the pairwise comparisons would be significantly elevated. In addition, the tumor incidences in the 35 and 175/100 mg/kg dose female mice fall within the spontaneous tumor incidence of this strain of mice from that time period (Parker et al., 2006; Haseman et al., 1985).
- . As indicated by Parker et al. (2006), the lack of treatment-related precursor lesions in the livers of the female mice supports the theory that the observed liver tumors were not induced by RDX.
- . The reported differences reported by Lish et al. (1984) and Parker et al., (2001 and 2006) in the number of female mice used in the RDX study, while not major, do undermine confidence in the overall quality of the study.
- . Because liver adenomas, carcinomas and adenomas combined with carcinomas are all analyzed separately, the experiment-wise error rate for type 1 error is well above 0.05 for liver tumors. No correction was made for multiple comparisons.

[I should mention that I agree with the EPA's decision to use the PWG revised tumor incidences for the female mice.]

- Male and female rats
The combined neoplastic nodules and hepatocellular carcinomas grouping was not significantly increased for either the male or female rats. While a statistically significant increase in hepatocellular carcinomas was seen for the male rats, it is modest in magnitude and is largely due to an increased incidence at the highest dose where considerable toxicity occurred. Due to what appears to be considerable toxicity, this dose may have exceeded the maximum tolerated dose and the panel should discuss whether this dose should be eliminated from the analysis. If so, the evidence for an increase in the liver tumors is weakened considerably. [It should be noted that the carcinoma incidences in male rats for two highest doses fall outside of the range seen in NTP studies for this period (Haseman et al., 1985). However, the control incidence is also at the very high end of the range seen as well. The combined tumor incidences fall within the historical range.]

Lung tumor data

The observed significant dose-related increases in lung tumors is largely driven by increases seen at the highest (175/100 mg/kg) dose, a dose at which extensive toxicity was seen in both the male and female mice. As indicated above, the committee should consider whether this dose has exceeded the MTD and whether it should be included in the dose analyses. If it is eliminated, I believe that only the combined alveolar/bronchiolar adenoma or carcinoma combined incidences will show a significant trend. None of the

doses are significantly increased when tested using a pair-wise comparisons.

The carcinoma (but not adenoma) incidences seen at the highest dose for the male and female mice exceed the historical control range. For the 3 lower doses, the increased incidences of lung adenomas and carcinomas (separately) fall within the historical range (with the possible exception of adenomas seen in the 35 mg/kg dose in females where the incidence was 14.1% vs. a high of 14% in NTP studies. Because of rounding, a definitive conclusion cannot be reached). Data were not available for the combined categories. According to Lish et al. (1984), the incidences of lung tumors in the treated animals fall within the historical control incidences of the test laboratory. The authors did not consider the increases to be biologically significant.

Because lung adenomas, carcinomas and adenomas combined with carcinomas are all analyzed separately, the experiment-wise error rate for type 1 error is well above 0.05 for liver tumors. No correction was made for multiple comparisons.

No significant increase was seen in male or female rats in the other studies.

3e. (ii) **Cancer-specific toxicity values** (Section 2.3.1). As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well- conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the [Lish et al. \(1984\)](#) study for this purpose scientifically supported and clearly described?

The draft assessment adequately lays out in general terms the rationale for performing a quantitative analysis of the data. It does not go into specifics but I don't believe that this is necessary in this particular document. The uncertainty of the data is mentioned but, as mentioned above, there are numerous issues about the Lish et al. (1984) study that are subject to interpretation. Of the chronic studies, I believe that re-evaluation of the female mouse data conducted by Parker et al. (2006) based on the Lish et al. (1984) study is the best one to use for quantitative analysis (if one is to be performed.). As indicated above (and in Section 1.2.5 of the document), the 1.5% incidence of liver tumors in the control B6C3F1 mice was unusually low. This was reported by the study authors as significantly lower than those in historical controls, and is lower than the incidences seen this strain by the NTP (mean 8%, range 0-20%). This unusually low control incidence could significantly influence the dose response modeling and result in a high slope value that over-estimates the cancer risks.

Mode of action – I agree that the mode of action for the observed carcinogenic effects is unknown. The majority of the genotoxicity data with RDX is negative. However, there are a number of studies that suggest that the MNX metabolite may be genotoxic. Unfortunately the quality of the studies does not allow firm decisions to be reached.

4c. **Oral slope factor for cancer** (Section 2.3.3–2.3.4). The draft assessment presents an overall

oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

The derivation of the oral slope factor seems to have been performed consistent with EPA policy. Combining the liver and lung data for the quantitative analysis is not something that I have seen previously but as indicated in the document, the approach appears to be consistent with EPA policy and supported by the NRC (1994; see page 230). On page D-3 of the Supplemental Information document it states, "If the BMDL estimates were "sufficiently close" (i.e., differed by threefold or less), then the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, then the lowest BMDL was selected as the POD." The use of the lowest BMDL should have some additional justification. I would think that the model with the best fit would be used, regardless of the derived BMDL estimate.

Because of the uncertainties associated with the Lish et al. (1984) study, particularly the unusually low incidence of liver tumors in the control animals, the OSF could easily be an overestimate of the risk. As a result, I am not sure it should be generated. If used, it used, the OSF should be put in brackets or marked with an asterisk or both each time that it is used so that the reader is aware of the uncertain nature of the RDX - cancer relationship that was used in its derivation.

Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Yes. I would suggest adding the following to indicate some of the uncertainty or limitations in the animal cancer bioassay results.

In the Summary, add "limited" to the following sentence as shown. Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on limited evidence of positive trends in liver and lung tumor incidence in experimental animals.

In the body, add some clarifying or cautionary language on page xxv, line 26. In spite of limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk... or Cognizant of limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk....

Dr. Joanne English

1. **Literature search/study selection and evaluation.** The section on Literature Search Strategy | Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Response: In general, the literature search strategy, and study selection and evaluation considerations appear to be well described and documented. One aspect that needs clarification is whether short term studies showing nervous system effects were appropriately considered for dose-response analysis, regardless of study duration. Specifically, on p. 29, second bullet, a justification for not extracting information from short-term studies into evidence tables is given:

- “Studies investigating the effects of acute/short-term and dermal exposures and case reports are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposure. “

However, elsewhere in the document it indicates that nervous system effects are as pronounced with short-term exposure compared with longer term exposure (noted on page 128, lines 23-24). As nervous system effects appear to lack appreciable chronicity and are being considered for derivation of an RfD, the short term studies identifying nervous system effects may be pivotal. Consider moving short term studies that are currently in the “Supplemental Studies” bin (Figure LS-1) into the “Sources of Health Effects Data” bin, and review accordingly.

With respect to scoping of the Toxicological Review, as it relates to study selection and evaluation considerations, it is notable that RDX is a poorly water soluble material that has been manufactured and/or formulated to different particle size ranges. The particle size range of RDX preparations is a toxicologically significant physical attribute, and differing RDX preparations with respect to particle size have been discussed within the document. It may therefore be appropriate to include particle size as an attribute in Table 1-1 (p. 42) of the chemical identity and physicochemical properties of RDX. As it has been acknowledged that particle size influences response/toxicity of RDX, the inference is that there may be RDX preparations (of size ranges that have not been evaluated toxicologically) that are outside of the scope of the review. To make clear which RDX substances are within or outside the scope of this risk assessment, it is suggested that the range of RDX particle sizes that are encompassed by this review be included Table 1-1. If the risk assessment is intended to address soluble RDX from environmental sources, it would be helpful to review the evidence that compares solubilized and particulate RDX. If specific particle sizes of RDX preparations are unknown, then qualitative descriptions (e.g., finely powdered to course granules) might be added to Table 1-1 or as a footnote to Table 1-1.

2. **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al., 2012a; Sweeney et al., 2012b).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

Response: The parent compound is likely the active moiety for nervous system effects, but for other identified health effects (kidney and other urogenital effects or testicular effects), it is unknown whether the parent compound or an active metabolite is responsible for the toxicity. The data are not sufficient to determine the toxicologically active moiety for kidney and other urogenital effects or testicular effects, therefore application of the conventional default approach (UF_H of 10x) is appropriate (WHO/IPCS Guidance in Chemical Specific Adjustment Factors, 2005).

With respect to the nervous system effects, the human model was optimized using blood concentrations from one child and 5 adult exposures where the exposure amounts are not known. It is unclear what different factor other than the default uncertainty factor (UF_H of 10x) might be available. How would such a factor reflect the heterogeneity and central tendency (WHO/IPCS Guidance in Uncertainty in Hazard Characterization, 2014) of the population at risk of exposure to RDX, and encompass suspected aggravating lifestyle factors, such as drinking alcohol and tobacco smoking?

3. Hazard identification and dose-response assessment. In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system-specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) to reach the following conclusions.

[Note: As suggested by the Chemical Assessment Advisory Committee panel that reviewed the draft IRIS assessment of benzo[a]pyrene, the charge questions in this section are organized by

health outcome, with a question on each hazard identification followed by questions on the corresponding organ/system-specific toxicity values. This suggestion, however, entails some redundancy, as some questions apply equally to multiple health outcomes.]

3a. Nervous system effects

- Nervous system hazard (Sections 1.2.1, 1.3.1). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion.

Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

Response: The available studies clearly support the conclusion that nervous system toxicity is a human hazard of RDX exposure.

- Nervous system-specific toxicity values (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

Response: Nervous system effects appear to be equally or more pronounced with short-term exposure compared with longer term exposure, (noted on page 128, lines 23-24) suggesting possible temporal effects on dynamics (e.g., up or down regulation of genes involved in neurotransmission; development of tolerance) or kinetics (e.g, induction of metabolic clearance), or age-related sensitivities. This raises the question whether the sub chronic study (Crouse et al. 2006) is the most appropriate study for derivation of the reference value for nervous system effects, or if a shorter term study (e.g., Cholakakis et al. 1980) would be more sensitive.

- Points of departure for nervous system endpoints (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described?

Response: Selection of convulsions as an endpoint to represent nervous system toxicity in D-R modeling is problematic, and not consistent with EPA's general practice to "not develop reference values based on frank effects," as acknowledged on page 135, lines 20-21. Convulsions are a severe and life-threatening endpoint. In contrast, the chosen endpoint should be the most sensitive adverse nervous system effect or its immediate precursor. Other less severe manifestations of nervous system effects have been documented in humans and/or animal models in the absence of overt clinical signs (MacPhail et al. 1985), such as altered reflexes, hyperactivity, decreased motor activity, cognitive deficits, and tremors; one or more of these effects may be more appropriate as a basis for deriving a reference value, if possible. Where incidence data are lacking, consideration should be given to use of NOAELs or LOAELs as PODs.

Are the calculations of PODs for these studies scientifically supported and clearly described?

Response: If the incidence of convulsions is selected as the endpoint to represent the hazard to the nervous system, consider whether the incidence data from Crouse et al. and Cholakakis et al. studies might be pooled for the purpose of BMD modelling to improve the power of the analysis. Studies were conducted by the same method of administration (gavage) and using the same species (rat) and strain (SD). The duration of dosing differs, but because the convulsion endpoint is an acute or largely acute effect, the influence of dosing duration appears to be minimal to negligible. The argument was presented that the Crouse et al. study was better designed to identify nervous system effects; however, since the observation of convulsions is unequivocal and reported at a lower dose level in the Cholakakis et al. study, it is not clear how the study design differences would justify excluding use of the Cholakakis et al. data.

Is the calculation of the HEDs for these studies scientifically supported and clearly described?

Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk?

Response: EPA's benchmark dose technical guidance indicates that a BMR near the low end of the observable range is typically selected as the basis for obtaining BMDs and BMDLs to serve as potential PODs. The limit of sensitivity of the Crouse et al. study, with group sizes of 20 (after combining the sexes) is perhaps 5% at best. The rationale given for the selection of a 1% benchmark response level is the severity of the endpoint (convulsions), however, it is also acknowledged that uncertainty is increased by extrapolating from a POD that is outside of the observable range. It is unclear if these factors have been appropriately balanced in the choice of benchmark response level.

Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

(iv) **Uncertainty factors for nervous system endpoints (Section 2.1.3).** Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.¹

Response:

- **The application of the interspecies UF of 3x** is appropriate and clearly described, but stops short of characterizing the toxicokinetic differences between rat and human. For further transparency, consider reporting in the Toxicological Review the ratio of the rat/human dose metric identified by PBPK modeling (provided in the Supplement, p. C-29), which is then applied to determine the PODHED.
- **The application of the intraspecies UF of 10x:** Referring to question 2c (above): "In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead? At this stage, it is unclear what factor other than the default uncertainty factor might be available that would reflect the heterogeneity and central tendency (WHO/IPCS Guidance in Uncertainty in Hazard Characterization, 2014) of the population at risk of exposure to RDX,

and encompass suspected aggravating lifestyle factors, such as drinking alcohol and tobacco smoking. Further comment on the application of intraspecies UF of 10x will be reserved until preliminary SAB responses to question 2c and related discussion are available.

- **The application of the subchronic to chronic UF of 1:** The data appear to support a lack of chronicity for nervous system effects, and support the subchronic to chronic UF of 1x. The reasons for applying the sub chronic to chronic UF of 1 to all PODs was justified on page 128, beginning on line 22. As stated on lines 23 - 25: “(1) in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in chronic studies, and (2) other studies upon which a POD was derived were of 2-year duration.”

The document continues to discuss support for the selection of the subchronic to chronic UF of 1 but compares studies involving differing methods of administration (gavage versus dietary), which muddies the argument; see lines 32 - 36 (reproduced below):

“chronic dietary doses associated with convulsions were ≥ 35 mg/kg-day and were at least fourfold higher than gavage doses that induced convulsions in 14- and 90-day studies (i.e., 2 mg/kg-day in Cholakakis et al. (1980) and 8 mg/kg-day in Crouse et al. (2006) (also see Table 1-3 and Figure 1-1). This may be due to differences between dietary and gavage administration (see Sections 2.1.1 and 2.1.7).”

Rather than comparing dietary and gavage studies of differing durations to support a sub chronic UF of 1x, the rationale might be strengthened by comparing studies of differing durations where the same method of administration was employed, as well as the same species and ideally strain. For example, Angerhofer et al., 1986, MacPhail et al., 1985; and Schneider et al. 1978 all used gavage dosing of SD rats (for 9 days, 30 days, and 90 days, respectively). No overt effects were reported in rats given RDX at 10 mg/kg-day for 9 or 30 day treatments. Similar levels of lethality were obtained at 20 mg/kg-day for 90-days (~27%) and 9 days (31%). Likewise Cholakakis et al. (1980) and Crouse et al. (2006) used gavage dosing of F344 rats for 2 weeks and 13 weeks, respectively. Considering the dose spacing, LOAELs for nervous system effects were reasonably similar in these studies; 2 and 8 mg/kg-day, respectively. These comparisons indicate a lack of chronicity for nervous system effects. Additionally on lines 31-32, it states: “The available bioassays suggest that chronic exposure would not lead to effects at lower doses than those induced by subchronic exposure.” and that a UF of 1 was applied to PODs derived from studies of less-than-chronic duration. Clarify that this sentence is specific to the nervous system toxicity endpoint.

To further strengthen the rationale for the 1x sub chronic to chronic uncertainty factor, consider discussing evidence pertaining to the potential for tissue accumulation.

- **The application of the LOAEL to NOAEL UF of 1** is appropriate and clearly described.
- **The database uncertainty factor of 3x** is proposed to account for inadequacies in the database for characterizing neurotoxicity hazard. In the document, several additional studies and measurements are suggested to reduce the uncertainty in the neurotoxicity database, including a suggestion to conduct developmental neurotoxicity studies. Comment on the proposed application of database UF of 3x will be reserved until preliminary SAB responses and discussion related to the neurotoxicity endpoint and database are available.

Clarifying question - why does the composite UF depicted by the shaded bar in figure 2-2 for the Crouse et al. and Cholakakis et al. studies appear to be > 100x? Rounding artifact?

- Nervous system-specific reference dose (Section 2.1.4). Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

3b. Kidney and other urogenital system effects

. (i) Kidney and other urogenital system hazard (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

. (ii) Kidney and other urogenital system-specific toxicity values (Section 2.1.1). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

. (iii) Points of departure for kidney and other urogenital system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

Response: Since the mode-of action for kidney and other urogenital system effects is unknown, and the toxic moiety of RDX is unknown, it is unclear that the modeled internal dose metric (RDXAUC) was appropriate for deriving human equivalent oral exposures.

. (iv) **Uncertainty factors for kidney and other urogenital system endpoints (Section 2.1.3).** Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Response:

- **The application of the interspecies UF of 3x** is clearly described; the UF was reduced from 10x to 3x because PBPK modeling was used to derive a human equivalent dose thus accounting for the toxicokinetic interspecies uncertainty. Since the mode-of action for kidney and other urogenital system effects is unknown, and the toxic moiety of RDX is unknown, it is unclear that the modeled internal dose metric (RDXAUC) was appropriate for deriving human equivalent oral exposures. However, whether the PODHED is derived from PBPK modeling or alternatively, from body weight scaling to the 3/4 power (i.e., $BW^{3/4}$), following the hierarchy recommended in EPA guidance (2011), the UF of 3x is appropriate.
- **The application of the intraspecies UF of 10x:** Referring to question 2c (above): "In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?" At this stage, it is unclear what factor other than the default uncertainty factor might

be available that would reflect the heterogeneity and central tendency (WHO/IPCS Guidance in Uncertainty in Hazard Characterization, 2014) of the population at risk of exposure to RDX. Further comment on the application of intraspecies UF of 10x will be reserved until preliminary SAB responses to question 2c and related discussion are available.

- **The application of the subchronic to chronic UF of 1:** the reasons for applying the sub chronic to chronic UF of 1 to all PODs was justified on page 128, beginning on line 22. As stated on lines 23 - 25: "(1) in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in chronic studies, and (2) other studies upon which a POD was derived were of 2-year duration." The Levine et al. 1983 study was of 2-year duration; therefore the application of the UF of 1x for kidney and urogenital system effects was appropriate.
Additionally on lines 31-32 it states: "The available bioassays suggest that chronic exposure would not lead to effects at lower doses than those induced by subchronic exposure." and that a UF of 1 was applied to PODs derived from studies of less-than-chronic duration. Clarify that this sentence is specific to the nervous system toxicity endpoint.
- **The application of the LOAEL to NOAEL UF of 1** is appropriate and clearly described.
- **The database uncertainty factor of 3x** is proposed to account for inadequacies in the database for characterizing neurotoxicity hazard. In the document, several additional studies and measurements are suggested to reduce the uncertainty in the neurotoxicity database, including a suggestion to conduct developmental neurotoxicity studies. Comment on the proposed application of database UF of 3x will be reserved until preliminary SAB responses and discussion related to the neurotoxicity endpoint and database are available.
 - (v) Kidney and other urogenital system-specific reference dose (Section 2.1.4). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

3c. Developmental and reproductive system effects

(i) Developmental and reproductive system hazard (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

Response: In Section 1.2.3, pertaining to developmental effects of RDX, the document concludes on p. 91 (lines 8 - 11):

"Developmental studies in rats (Angerhofer et al., 1986; Cholakakis et al., 1980) demonstrated effects on offspring survival, growth, and morphological development only at doses associated with severe maternal toxicity and mortality. No dose-related developmental effects were observed in rabbits (Cholakakis et al., 1980)."

Clarify if the statistically significant, dose-related decreasing trends in fetal body weight and length in SD rats administered RDX by gavage from GD 6 to 15 (Angerhofer et al., 1986), when appropriately analyzed by EPA on a per litter basis, shows that embryo toxicity is expressed at non-maternally toxic dose levels. Was a similar trend test performed for the fetal body weights in the rabbit developmental toxicity study (Cholakis et al. 1980)? These data may be suggestive of a selective embryotoxic effect, particularly if displayed in both species evaluated.

. (ii) Reproductive system-specific toxicity values (Section 2.1.1). Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?

. (iii) Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

. (iv) **Uncertainty factors for reproductive system endpoints (Section 2.1.3).** Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Response:

- **The application of the interspecies UF of 3x** is appropriate and clearly described; it was reduced from 10x to 3x because allometric scaling was used to derive a human equivalent dose thus accounting for the toxicokinetic interspecies uncertainty.
- **The application of the intraspecies UF of 10x:** Referring to question 2c (above): “In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?” At this stage, it is unclear what factor other than the default uncertainty factor might be available that would reflect the heterogeneity and central tendency (WHO/IPCS Guidance in Uncertainty in Hazard Characterization, 2014) of the population at risk of exposure to RDX. Further comment on the application of intraspecies UF of 10x will be reserved until preliminary SAB responses to question 2c and related discussion are available.
- **The application of the subchronic to chronic UF of 1:** the reasons for applying the sub chronic to chronic UF of 1 to all PODs was justified on page 128, beginning on line 22. As stated on lines 23 - 25: “(1) in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in chronic studies, and (2) other studies upon which a POD was derived were of 2-year duration.” The Lish et al. 1984 study was of 2-year duration; therefore the application of the UF of 1x for testicular effects was appropriate. Additionally on lines 31-32 it states: “The available bioassays suggest that chronic exposure would not lead to effects at lower doses than those induced by subchronic exposure.” and that a UF of 1 was applied to PODs derived from studies of less-than-chronic duration. Clarify that this sentence is specific to the nervous system toxicity endpoint.
- **The application of the LOAEL to NOAEL UF of 1** is appropriate and clearly described.
- **The database uncertainty factor of 3x** is proposed to account for inadequacies in the database for characterizing neurotoxicity hazard. In the document, several additional studies

and measurements are suggested to reduce the uncertainty in the neurotoxicity database, including a suggestion to conduct developmental neurotoxicity studies. Comment on the proposed application of database UF of 3x will be reserved until preliminary SAB responses and discussion related to the neurotoxicity endpoint and database are available.

(v) Reproductive system-specific reference dose (Section 2.1.4). Is the organ/system- specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

3d. Other noncancer hazards (Sections 1.2.4, 1.2.6, 1.3.1). The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision. Are other non-cancer hazard adequately described?

Response: General comment. With respect to the database for RDX, the document concludes that “Overall, while the RDX database adequately covers major systemic effects, including reproductive and developmental effects, uncertainties in the adequacy of the database were identified in characterization of the neurotoxicity hazard.” (page 130, lines 26-28). The database then, appears to be quite strong, with the possible exception of weaknesses in the characterization of developmental neurotoxicity and/or subclinical neurotoxicity. Therefore, since major systemic effects are adequately covered, it may be incongruent to draw “no conclusions” regarding “other non cancer” effects as human hazards of RDX exposure. With the evidence at hand, isn't it possible to conclude that the studies reviewed do or do not provide convincing evidence that the effect in question is a human hazard of RDX, when environmental exposure scenarios within the scope of the assessment are considered?

Section 1.2.4. Liver effects: no conclusion drawn as to whether liver effects are a human hazard of RDX.

Section 1.2.6. Other non cancer effects: no conclusions are drawn regarding the other noncancer effects as human hazards of RDX exposure. The other noncancer effects discussed in the Supplemental Information (Section C.3.2) are: ocular effects, cardiovascular effects, musculoskeletal effects, immune system effects, gastrointestinal effects, hematological effects.

Are other non-cancer hazard adequately described?

Response: Not addressed in either the Toxicological Review or the Supplemental Information were the dose-related effects on body weights and/or body weight gains, although this was identified as a potential adverse effect of RDX elsewhere (e.g., Sweeney et al. 2012; EPA, 2012). Dose-related decreases in body weight gain were frequently observed in repeated dose studies. It may be appropriate to identify this effect as a hazard of RDX exposure, reflecting generalized systemic toxicity. It may also be appropriate to carry this effect forward in the dose-response analysis. Include a discussion of the evidence for body weight effects associated with RDX exposure.

Section 1.3.1. Effects other than cancer

This section states that “evidence for developmental toxicity and liver toxicity was more limited” than that for the nervous system, urogenital, and reproductive endpoints (page 113, line 18).

With respect to developmental toxicity, the document states “In animal studies, developmental effects, including offspring survival, growth, and morphological development, were observed only at doses associated with maternal mortality (Angerhofer et al., 1986; Cholakakis et al., 1980)” see page 113, lines 19-21). Address the possibility of a selective embryo toxic effect of RDX. Specifically, the statistically significant, dose-related decreasing trends in fetal body weight and length in SD rats administered RDX by gavage from GD 6 to 15 (Angerhofer et al., 1986), when appropriately analyzed by EPA on a per litter basis, suggest that embryo toxicity is expressed at non-maternally toxic dose levels. Also, it is unclear whether there is a similar decreasing trend in fetal body weights in the rabbit developmental toxicity study (Cholakakis et al. 1980).

3e. Cancer

. (i) Cancer hazard (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

. (ii) Cancer-specific toxicity values (Section 2.3.1). As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, “When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well- conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.” Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

. (iii) Points of departure for cancer endpoints (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

4. Dose-response analysis. In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) in the following analyses.

4a. Oral reference dose for effects other than cancer (Sections 2.1.5–2.1.8). The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous

system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

4b. Inhalation reference concentration for effects other than cancer (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

4c. Oral slope factor for cancer (Section 2.3.3–2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

4d. Inhalation unit risk for cancer (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Response: The major conclusions of the draft assessment appear clearly and appropriately presented in the Executive Summary. As preliminary SAB responses and related discussion becomes available, and changes to the body of the draft assessment are recommended by the SAB-CAAC, additional comments may be developed.

References

- Cholakakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-C-8027). Kansas City, MO: Midwest Research Institute.
- Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Subchronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.
- Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM. (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report--phase V. Chicago, IL: IIT Research Institute.
- Lish, PM; Levine, BS; Furedi, EM; Sagartz, JM; Rac, VS. (1984). Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes 1-

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Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a).

Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review]. Regul Toxicol Pharmacol 62: 107-114.

<http://dx.doi.org/10.1016/j.yrtph.2011.12.007>

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Regul Toxicol Pharmacol 64: 205-224. <http://dx.doi.org/10.1016/j.yrtph.2012.07.005>

Dr. Alan Hoberman

Developmental and reproductive system effects

- (i) **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

Male Reproductive Toxicity

This reviewer agrees that there is minimal suggestive evidence of male reproductive effects, specifically,

an increased incidence of testicular degeneration (10–11%) was observed in male B6C3F₁ mice exposed to ≥ 35 mg/kg-day RDX for 2 years in the diet compared to concurrent (0%) and historical (1.5%) controls ([Lish et al., 1984](#)).

An increased incidence of germ cell degeneration was observed in rats exposed to 40 mg/kg-day (40%) compared with controls at 12 months (0%); by 24 months, almost all male rats (including controls) had testicular masses (interstitial cell tumors), and no instances of germ cell degeneration

The lack of concordance with other studies ([Crouse et al., 2006](#); [Levine et al., 1990](#); [Levine et al., 1981a, b](#); [Hart, 1976](#)) or dogs ([Hart, 1974](#)), the lack of a dose response, the lack of a significant change in testicular weight and the time (2 yrs or 1 yr) required for this finding to develop makes the findings questionable.

Female Reproductive Toxicity

Exposure to doses of 16 mg/kg/day or less did not produce any reproductive toxicity. A dose of 50 mg/kg-day produced significant maternal and paternal toxicity including a 27% mortality rate in the females. At this dose level, non-statistically significant reductions in fertility occurred. Interestingly, mating of treated male rats with untreated female rats (dominant lethal assay) also produced lowered fertility after 15 weeks of exposure (1.5 spermatogenic cycles) at a dose of 50 mg/kg/day. This result does not rule out a female effect on fertility of RDX, but it does appear that these effects are limited to very high maternally toxic doses.

This reviewer recommends that genotoxic and mutagenic aspects of reproductive hazard be addressed, although in somatic cells there is little indication of genetic toxicity.

This reviewer recommends that follicular counts in the ovaries, may also help evaluate the potential direct effects on the ovary.

No evaluation of estrous cycle was conducted prior to mating to look for changes in female hormone levels.

Developmental Toxicity

The only two generational feeding study ([Cholakis et al., 1980](#)) reported decreases in offspring survival (including both stillborn pups and postnatal deaths through the age of weaning) following a clearly maternally toxic dose of 50 mg/kg/day. Lower doses were not toxic to the dams or offspring. Although pup weights were also reduced at 16 mg/kg in the diet on PND 25 – probably because of direct ingestion of the RDX.

In an oral gavage embryo fetal developmental (EFD) toxicity study in F344 rats ([Cholakis et al., 1980](#)) maternal (mortality up to 31%) and embryo/fetal (reduced fetal weight and resorption) occurred at 20 mg/kg/day. Although in SD rats ([Angerhofer et al., 1986](#)), only maternal toxicity and increased resorption at 20 mg/kg/day. No additional maternal or embryo/fetal toxicity including no abnormalities of the fetuses occurred at these doses or lower doses in either strain. Treatment in both of these studies starts on gestation day 6, while implantation is still in progress and ends on gestation day 15, prior to the closure of the hard palate. A longer dosage period as suggested for all current EPA and OECD guidelines, may have yielded more fetal toxicity, especially an effect on fetal weight. A search of the literature by this reviewer could find no other appropriate studies for evaluation.

Questions from the reviewer,

Angerhofer, 1986 – why was the fertility rate in the 60% range. Almost double the normal number of rats per group started on test.

One 100 %, single conceptus resorbed. in group two. Should be excluded from analysis. Fetal weight was reduced down to 2 mg/kg, no maternal toxicity at this dose. This makes 2 mg/kg the lowest effect level. Did not occur in first EFD at 2 mg/kg

Rabbits evaluated in an oral gavage EFD toxicity study ([Cholakis et al., 1980](#)) dosed on days 6 to 29 of gestation appear to be less sensitive than rats as exposures up to 20 mg/kg/day did not produce any maternal or embryo/fetal toxicity. The conclusion of apparent less sensitivity is difficult to support without knowing if exposure in the rabbit was equivalent to or more than that observed in the rat. The number of rabbits used per group was 11 or 12 making the power of this particular evaluation questionable.

1. **Reproductive system-specific toxicity values** (Section 2.1.1). Is the selection of the [Lish et al. \(1984\)](#) study that describes male reproductive system effects scientifically supported and clearly described?

Based on the data provided it is difficult to scientifically support the conclusion that male fertility has been affected by the test substance. There needs to be a confirmation of the histopathology of the testes (Lish et al., 1984 positive results and other study negative results) by experienced pathologist in testicular histopathology. The idea of pathology working group (PWG) for evaluation of the testes is suggested.

- (ii) **Points of departure for reproductive system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of a HED for this study scientifically supported and clearly described?

If one assumes that the effect on male fertility as noted in the 2 yr mouse study is a real effect than the extrapolation for the calculation of a POD is appropriate. However this reviewer is questioning the premise that the finding and therefore the POD would not make sense as calculated.

- (iii) **Uncertainty factors for reproductive system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

‘ Incidence data on testicular degeneration as reported by [Lish et al. \(1984\)](#) were amenable to modeling.”

Again the premise is that observed testicular degeneration was appropriate to select for dose-response analysis. [Lish et al. \(1984\)](#) is being questioned. This 2-year study: (1) included histopathological examination of male reproductive organs; (2) included four dose groups and a control group, and adequate numbers of animals per dose group (85/sex/group, with interim sacrifice groups of 10/sex/group at 6 and 12 months); and (3) reported individual animal data. While the criteria were met for the modeling, the results are still in question.

- (iv) **Reproductive system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

Male Reproductive Effects

- “ A single dataset for male reproductive effects, specifically the incidence of testicular degeneration as reported in male B6C3F₁ mice exposed to RDX in diet for 24 months ([Lish et al., 1984](#)), was brought forward for quantitative analysis. The RfD for male reproductive effects is based on this dataset.”

The premise for this finding is under question.

Embryo Fetal Developmental Effects

In an embryo fetal developmental (EFD) toxicity study in F344 rats ([Cholakakis et al., 1980](#)) maternal (mortality up to 31%) and embryo/fetal (reduced fetal weight and resorption) occurred at 20 mg/kg/day. Although in SD rats ([Angerhofer et al., 1986](#)), only maternal toxicity and increased resorption at 20 mg/kg/day. No additional maternal or embryo/fetal toxicity including no abnormalities of the fetuses occurred at these doses or lower doses in either strain. Fetal weights were reduced down to 2 mg/kg.

If the male reproductive effects are in question than the organ system reference dose should be made on the resorption and fetal weight data that occurred at 20 mg/kg/day.

Dr. Jacqueline Hughes-Oliver

1. **Literature search/study selection and evaluation.** The section on *Literature Search Strategy / Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations **including exclusion criteria**, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Hughes-Oliver: The approach seems appropriate and is clearly described.

2. **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans ([Sweeney et al., 2012a](#); [Sweeney et al., 2012b](#)).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported?
Are the uncertainties in the model appropriately considered and discussed?

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

3. **Hazard identification and dose-response assessment.** In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system-specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) to reach the following conclusions.

[Note: As suggested by the Chemical Assessment Advisory Committee panel that reviewed the

draft IRIS assessment of benzo[a]pyrene, the charge questions in this section are organized by health outcome, with a question on each hazard identification followed by questions on the corresponding organ/system-specific toxicity values. This suggestion, however, entails some redundancy, as some questions apply equally to multiple health outcomes.]

3a. Nervous system effects

- (i) **Nervous system hazard** (Sections 1.2.1, 1.3.1). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. **Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?**
- (ii) **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the [Crouse et al. \(2006\)](#) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?
- (iii) **Points of departure for nervous system endpoints** (Section 2.1.2). **Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described?** Are the calculations of PODs for these studies scientifically supported and clearly described? **Is the calculation of the HEDs for these studies scientifically supported and clearly described?** Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

Hughes-Oliver: The report argues quite effectively that convulsions are indicative of severe neurological effects, and hence warrant the more stringent protections offered by controlling for a benchmark response level of 1% extra risk. Dose-response modeling clearly documents a significant increasing relationship between incidence of convulsions and dose levels, thus supporting selection of convulsions as a relevant endpoint to represent hazard to the nervous system. The calculations of BMD and BMDL from many available models are appropriate and clearly described. The details provided in Appendix D allow readers to replicate analyses and arrive at the same conclusions. Statistical science is not unanimous in how one should choose from among several candidate models, but the approach used here is reasonable and clearly explained. The calculations of candidate PODs based on rat studies are scientifically supported

and clearly described. There are a few typos. In Table 2-2 on page 1-7 of Chapter 2, the selected model for the Crouse et al (2006) data is a multistage 3^o, not a multistage 2^o (see Table D-5 on page D-9 in Appendix D). In Table D-3 on page D-4 of Appendix D, in "Basis for model selection," replace "the multistage 5^o model was selected" with "the multistage 2^o model was selected." The text on line 7 of page D-6 should be deleted. In Table D-4 on page D-6, the multistage 2^o model does not have the lowest AIC among multistage models, not according to numbers reported in the table. The Cholakakis et al (1980) data of Table D-1 on page D-2 should be "18/25 (72%)" not "18/24 (75%)". These typos do not affect other results and are mentioned here only to improve clarity.

The discussion on lines 6-11 of page 1-4 in Chapter 2 is confusing. Section 2.1.1 on identification of studies for dose-response analysis concerning nervous system effects begins by saying convulsions were reported in seven studies that are summarized in Table 2-1. Strengths and weaknesses of these seven studies are then discussed, ending at the top of page 1-4. However, reference is made in lines 6-11 to studies not included in Table 2-1. For example, the 13-week mouse study by Cholakakis et al (1980) does not appear to have reported convulsions (see page 1-10 of Chapter 1), so discussing it here is confusing. On the other hand, the single-dose 6-week dog study by von Oettingen et al (1949) is discussed but was included in Table 2-1, even though it did mention convulsions (see page 1-12 of Chapter 1). Likewise, both studies by Angerhofer et al (1986) (pages 1-12 and 1-13 of Chapter 1) mention convulsions and so appear to be reasonable candidates for inclusion in Table 2-1.

- (iv) **Uncertainty factors for nervous system endpoints** (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.²
- (v) **Nervous system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

¹ Note that the database uncertainty factor applies to each of the hazards identified in the toxicological review.

3b. Kidney and other urogenital system effects

- (i) **Kidney and other urogenital system hazard** (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. **Are all hazards to kidney and urogenital system adequately assessed?** Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?
- (ii) **Kidney and other urogenital system-specific toxicity values** (Section 2.1.1). Is the selection of the [Levine et al. \(1983\)](#) study that describes kidney and other urogenital system effects scientifically supported and clearly described?
- (iii) **Points of departure for kidney and other urogenital system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? **Is the calculation of the HED for this study scientifically supported and clearly described?**

Hughes-Oliver: The calculations of BMD and BMDL from many available models are appropriate and clearly described. The details provided in Appendix D allow readers to replicate analyses and arrive at the same conclusions. Statistical science is not unanimous in how one should choose from among several candidate models, but the approach used here is reasonable and clearly explained. The calculation of a candidate POD based on the rat study is scientifically supported and clearly described.

- (iv) **Uncertainty factors for kidney and other urogenital system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?
- (v) **Kidney and other urogenital system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

3c. Developmental and reproductive system effects

- (i) **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects

associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these **decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?**

- (ii) **Reproductive system-specific toxicity values** (Section 2.1.1). Is the selection of the [Lish et al. \(1984\)](#) study that describes male reproductive system effects scientifically supported and clearly described?
- (iii) **Points of departure for reproductive system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? **Is the calculation of the HED for this study scientifically supported and clearly described?**

Hughes-Oliver: The calculations of BMD and BMDL from many available models are appropriate and clearly described. The details provided in Appendix D allow readers to replicate analyses and arrive at the same conclusions. Statistical science is not unanimous in how one should choose from among several candidate models, but the approach used here is reasonable and clearly explained. The calculation of a candidate POD based on the mouse study is scientifically supported and clearly described. However, the report should explain the use of dose value 107 in Table D-1 on page D-2. While describing the study in Table 1-3 on page 1-10, it was mentioned that the original highest dose of 175 was changed in week 11 to 100 due to excessive mortality. How was the value 107 determined from 175 and 100?

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- (iv) **Uncertainty factors for reproductive system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?
- (v) **Reproductive system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

3d. **Other noncancer hazards** (Sections 1.2.4, 1.2.6, 1.3.1). The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this **decision. Are other non- cancer hazard adequately described?**

3e. **Cancer**

- (i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.
- (ii) **Cancer-specific toxicity values** (Section 2.3.1). As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well- conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the [Lish et al. \(1984\)](#) study for this purpose scientifically supported and clearly described?

- (iii) **Points of departure for cancer endpoints** (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

Hughes-Oliver: The calculations of PODs and oral slope factors appear to be consistent with EPA's guidelines, but these sections are much less explanatory than other sections in the report. Several places in the report (e.g., page 1-26 line 30 of Chapter 2) mention comparing survival curves across dose groups, but these curves are never shown, nor is there data presented to allow calculation of these curves. (Presumably, the curves were compared in the Lish et al 1984 paper, but not repeated by the EPA?) The MS-COMBO procedure is not fully explained.

4. **Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) in the following analyses.

- 4a. **Oral reference dose for effects other than cancer** (Sections 2.1.5–2.1.8). The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on

nervous system effects as described in the [Crouse et al. \(2006\)](#) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by [Cholakakis et al. \(1980\)](#) that is lower (by approximately fivefold) as described in Section 2.1.4?

Hughes-Oliver: The selection process is clearly described, but there are several typos while discussing/presenting the analysis of mortality. Table D-14 on page D-28 argues that the Multistage 3rd model should be selected for the Angerhofer et al 1986 study because it has the lowest BMDL at 0.588; but in fact the Gamma model has the lowest BMDL at 0.538, so its information belongs in Table 2-6 on page 1-20 of Chapter 2, with a BMD of 5.1, thus making 2.1 the smallest BMD in that table and will change references to the range that appears on page 1-21. Table 2-6 on page 1-20 of Chapter 2 says the 13-week rat study by von Oettingen et al 1949 is not amenable to modeling, and page 1-21 incorrectly says the same study had mortality only at the highest dose; a more accurate description is that none of the models **available in software BMDS** provide a reasonable fit to the data, as discussed in Table D-12 on page D-25. In Table D-7 on page D-13, the logistic (not the log-logistic) model did not

achieve an adequate fit. Page D-22, line 11: Levine et al 1981b, not 1983. These typos do not change the qualitative conclusions of Sections 2.1.6 and 2.1.7, that the inclusion of mortality would not significantly change the oral reference dose.

The methods used to derive the 95% confidence intervals shown in Table 2-6 on page 1-20 should be explained.

While Sections 2.1.4 and 2.1.5 clearly explain the rationale for selecting the oral reference dose based on the Crouse et al 2006 study, and not the Cholakakis et al 1980 study, I have reservations regarding this selection. The fivefold difference in candidate reference values is too big to ignore. A minimal acknowledgement of this difference may be to declare pregnant animals as a potentially susceptible subgroup.

4b. **Inhalation reference concentration for effects other than cancer** (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

4c. **Oral slope factor for cancer** (Section 2.3.3–2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

Hughes-Oliver: Assuming that the MS-COMBO procedure truly provides benefits beyond separately modeling tumor types, then it makes sense that the overall oral slope factor should be determined from the combined analysis of both tumor types. It is interesting and a bit surprising that analysis of the same Lish et al 1984 data would lead to an overall oral slope factor of 0.11 in the existing IRIS entry but only 0.038 in this draft.

4d. **Inhalation unit risk for cancer** (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

5. **Executive summary.** Does the executive summary clearly and **adequately** present the major conclusions of the assessment?

Dr. Susan Laffan

3c. Developmental and Reproductive System Effects

- (v) *Developmental and reproductive system hazard (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?*

Male Reproductive Toxicity:

The evidence for direct male reproductive effects is very weak such that is questionable whether the weight of evidence supports this conclusion. Many of the reasons are already identified in the report – lack of effect in several studies of varying duration in rat and also in dog, and inconsistent testicular weight data with small magnitude changes. This finding was only in one species after a long duration of treatment, in a situation in which the animals were experiencing other marked toxicities. For instance, the high dose level was decreased after about 3 months of dosing due to excessive mortality and there is a difference in absolute and relative changes for testicular weight reported which implies there was a body weight effect at this dose. It is demonstrated in the rat that degeneration of Stage VII pachytene spermatocytes can occur after 20-30% food restriction for six weeks duration [Rehm 2006]. Additionally consider that in 2 year dietary mouse study, there was a lack of dose response in the apparently affected dose groups; the incidence of testicular degeneration was 0, 3.3, 3.2, 10, 11% in the control, 1.5, 7, 35 and 175/100 mg/kg/day groups, respectively. After two years of treatment the testicular findings were still at a low incidence (10-11%) and were not accompanied by tubular vacuolation (which typically reflects primary effects on the Sertoli cell or be secondary to loss of degenerating germ cells from the Sertoli cell cytoplasm), nor culminated in tubular atrophy [Creasy 2011]. The spermatogenic cycle in a mouse is approximately 33 days, so if this were a direct testicular toxicant several cycles would have been exposure and the effect would be expected to worsen with continued exposure. The other species were also treated through several spermatogenic cycles without an effect.

Questions from reviewer:

I was unable to obtain the full report for the mouse 2 year dietary study; do we have access to testicular histology data from the interim termination at 6 months and 12 months? Also, was the testicular degeneration graded by the pathologist; i.e. was it considered mild, moderate or severe?

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Were they body weight and food consumption effects? This information would be very useful data to put the findings in context.

Page 1-39, Line 35 - Please confirm whether rat testis at 24 months were histologically evaluated. Report states that testes were not evaluated due to masses in nearly all control and RDX treated rats. It is unclear why the draft report states that there were no instances of germ cell degeneration identified at 24 months.

Table 1-9: Levine (1983) study the table footnotes the testes germ cell degeneration finding with a description that there was testicular atrophy, it is unclear if there is a mix of degeneration and atrophy noted.

Female Reproductive Toxicity:

I agree there is no suggestive evidence that RDX affect female fertility in animal models as the non-statistically significant reductions in fertility only occurred at toxic dose level.

Development Toxicity:

None of the developmental studies in the rat, rabbit conducted reported a teratogenic hazard despite evaluating dose levels that were occasionally maternally toxic. This is an important piece of information for the overall risk assessment. The developmental toxicity observed was that typical of findings associated with maternal toxicity and occurred at maternally toxic dose levels. It is generally understood that maternal toxicity, evidenced by body weight loss or reductions in body weight gain and/or decreases in food consumption, can contribute to developmental toxicity of the fetus in animal models. Developmental toxicity associated with maternal toxicity typically manifests as fetal weight reductions, increases in post-implantation loss (i.e., embryofetal death), and increases in the incidence of certain fetal skeletal variations. There is recognition within the scientific community of the possible effects on the fetus from maternal toxicity in common animal models [Carney and Kimmel, 2007; Rogers, 2005]. This concept was the primary topic discussed in an International Life Sciences Institute-Health and Environmental Sciences Institute (ILSI-HESI) sponsor working group, and the proceedings have been published [Beyer, 2011]. The findings in the RDX developmental toxicity studies of increased post-implantation loss, decreased fetal body weight and fetal skeletal variations are those considered typically associated with maternal toxicity and occurred at maternal toxic dose levels.

The rabbit embryofetal development study at 20 mg/kg/day did have an apparent increase in fetal malformations at a low incidence which is difficult to put in context without a robust historical control database, systemic exposure levels and with a smaller group size of 10-11 litters. Additionally, the report (Cholakis, 1980, p20) notes that the maternal rabbits at 20 mg/kg/day gained less weight, so there was some maternal toxicity at the high dose tested. However, this is not necessarily an associated factor for the possible malformations. The report defends that the findings were not statistically significant and thus RDX was not considered specifically teratogenic in rabbits.

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Other Comments:

- Page 1-45, Figure 1-3: It may be a matter of rote procedure, but the decision to highlight only statistically significant findings in the exposure-response array is deceptive because the two studies identified with statistical significant findings (Levine 1990 and 1983) were not considered meaningful results, but the nonstatistically significant finding in Lish 1984 is the paramount finding for male repro effects and is not highlighted.
- Reconsider the use of term 'offspring survival' to categorize prenatal mortality as this term is more commonly associated with postnatal outcome.
- Figure1-4: typo in spelling of 'significantly' in the key

(vi) ***Reproductive system-specific toxicity values*** (Section 2.1.1). *Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?*

Based on the reasons provided above, the selection of the Lish 1984 study as an exemplar of male reproductive system effect is not robustly supported.

The remaining related charge questions will be answered after we establish consensus on these first two questions.

Dr. Lawrence Lash

1. **Literature search/study selection and evaluation.** The section on *Literature Search Strategy, Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations **including exclusion criteria**, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search involves two types of searches, one set of searches in the published literature, using databases such as PubMed, Toxline, Toxcenter, and Toxic Substances Control Act Test Submissions (TSCATS), and the other involving searches of unpublished studies conducted by the Department of Defense (DoD) using the Defense Technical Information Center (DTIC) database. With regard to these two types of searches, the first is fairly standard. In contrast, the use of non-published and non-peer reviewed studies would seem to go against normal practice. In the case of RDX, however, it is somewhat understandable that the DoD might conduct their own studies, although I would still expect some type of peer-review even if results were not to be published in the typical scientific literature. This is not a major concern, as less than 10% of the total number of studies retrieved from the various literature searches (i.e., 84 out of 1248) are from the DTIC database. Nonetheless, some comment should be added to the EPA document about the use of this database. For example, it could be noted that despite its lack of external peer review, this is a database of unique studies that exist nowhere else.

Additional details about search criteria and results are provided in an Appendix. I frankly did not find the Appendix to be very useful and would have preferred to maybe have had just a little more detailed explanation in the main document.

Inclusion and exclusion criteria for judging the appropriateness of references to be included in the analysis are clearly explained. In particular, I found the tables in this section to be very useful summaries of the pertinent information. This included Table LS-1 ("Inclusion-exclusion criteria for health effect studies"), Table LS-2 ("Studies determined not to be informative because of significant issues with design, conduct, or reporting"), and Table LS-3 ("Considerations and relevant experimental information for evaluation of experimental animal studies").

I do not have other studies to suggest that should be considered.

3. **Hazard identification and dose-response assessment.** In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system- specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) to reach the following conclusions.

3b. **Kidney and other urogenital system effects**

(i) **Kidney and other urogenital system hazard** (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. **Are all hazards to kidney and urogenital system adequately assessed?** Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

Although the sections on kidney and other urogenital system hazards would seem to be presented in a methodical manner, there are several concerns with the underlying logic of the presentation. In answer to the first question about whether all hazards are adequately assessed, my response would **No**. The reason for this is that data on renal papillary necrosis and sex-dependent differences in susceptibility are not properly assessed. With regard to the selection of suppurative prostatitis as an endpoint, the EPA proposes to use this as a surrogate for all kidney and urogenital system hazard. There are a couple of concerns with this approach. First, there are no reports of prostatitis in humans, only in rats. Second, there are issues with how one designates the form of prostatitis. Third, such an endpoint would of course not take into account the risk for females. Fourth, there is no known underlying mechanistic basis for linking nephrotoxic or other renal adverse effects with prostatitis. Fifth, the dose-response for suppurative prostatitis is not particularly straightforward (see comments next section). Thus, it is recommended that prostatitis be evaluated as a separate response from kidney effects.

One aspect of section 1.3.1 with which I have some concerns is the presentation of the mechanism by which RDX likely affects the kidneys and urogenital system. References are cited in support of the involvement of the GABA_A receptor, but the level of detail and clarity of the supporting data regarding this are not very clear from the presentation.

(ii) **Kidney and other urogenital system-specific toxicity values** (Section 2.1.1). Is the selection of the [Levine et al. \(1983\)](#) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

In presenting the dose-response analysis for the RfD based on kidney or other urogenital system effects, the lists of studies and key factors in each study that serve as the basis for this analysis are nicely presented in both text and table forms. The two issues that seem to be most important in defining the appropriateness of potential studies are route of oral administration (i.e., gavage vs. diet) and RDX particle purity. Clearer statements about these factors should be provided. Because of the dose-response, it would not seem that BMDL modeling is appropriate. Rather, use of a NOAEL seems more appropriate based on the database.

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(v) **Kidney and other urogenital system-specific reference dose** (Section 2.1.4).

Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

This section has a very nice summary table (Table 2-4 on page 1-16) and clearly explains the limitations of the database with kidney/urogenital system for derivation of the organ-specific RfD. Rationale is clear and the document states that the RfD based on this organ is of low confidence whereas that based on nervous system effects is of medium confidence. It is interesting that despite the difference in confidence level, the RfDs based on kidney/urogenital system and nervous system differ by very little (2×10^{-3} vs. 3×10^{-3} mg/kg-d, respectively). This point may be worth noting. In contrast, the RfD based on male reproductive toxicity (2×10^{-2} mg/kg-d) is also said to have a low level of confidence but is an order of magnitude different from the two other organ-specific RfD values.

As discussed above, however, the choice of suppurative prostatitis as a surrogate endpoint for all kidney and urogenital system hazard is deemed as inappropriate.

Recommendations: First, the EPA needs to provide a more thorough rationale for calculating organ-specific RfDs. While it is recognized that the standard approach is to use the most sensitive endpoint for calculation of an RfD value, there is nothing intrinsically wrong with calculating these so-called organ-specific values. However, it is unclear how the EPA intends for these different values to be used. Second, the RfD for suppurative prostatitis needs to be recalculated using a NOAEL approach, rather than BMDL modeling.

5. **Executive summary.** Does the executive summary clearly and **adequately** present the major conclusions of the assessment?

Overall, I found the Executive Summary to be clear and concise. This section is subdivided into sections as follows: 1) noncancer effects observed following oral exposure; 2) oral reference dose (RfD) for effects other than cancer; 3) noncancer effects observed following inhalation exposure; 4) inhalation reference concentration (RfC) for effects other than cancer; 5) evidence for human carcinogenicity; 6) quantitative risk of carcinogenic risk from oral exposure; 7) quantitative risk of carcinogenic risk from inhalation exposure; 8) susceptible populations and lifestages for cancer and noncancer outcomes; and 9) key issues addressed in the assessment.

For each subsection, the key points are clearly discussed. The Executive Summary also contains two simple, very informative tables.

A couple of additional points that I would suggest briefly adding are:

- 1) The main criteria used for choosing the principal study; and
- 2) The importance of RDX purity in published studies.

Dr. Stephen Lasley

Literature search/study selection and evaluation

In general, the literature search process is well described, thorough, and clearly documented. The search strategy, study selection considerations including exclusion criteria, and evaluation mechanisms are appropriate and well defined. There are no apparent sources of additional peer-reviewed studies that should be considered.

Study results are evaluated using quality considerations that addressed aspects of design, conduct, or reporting that could affect the interpretation of findings, overall contribution to the synthesis of evidence, and determination of hazard potential according to EPA guidelines. The purpose is to identify the stronger, more informative studies based on this uniform evaluation of quality characteristics across investigations of similar design.

Evaluation of the experimental animal database for RDX is limited to studies of oral toxicity. A well defined set of studies provided useful information on RDX toxicity, including an array of endpoints for neurotoxicity and immunotoxicity. A secondary set of studies provided information relevant to RDX toxicokinetics and mechanism of action on the nervous system, but not health effects data.

Hazard Identification – 3a(i). Nervous system hazard

It is clearly apparent that the available research evidence supports the assessment conclusion that nervous system toxicity is a human hazard of RDX exposure. In a cross-sectional study neurobehavioral function was impaired in Chinese workers occupationally exposed to RDX (Ma and Li, 1993). Memory retention and block design scores were significantly lower among exposed workers compared to unexposed workers from the same plant. These types of effects are more sensitive to low levels of exposure than seizures, and are typical of the reported actions of many more common environmental agents. However, this study did not consider potential confounders, and there was limited information characterizing RDX exposure, thus significantly diminishing the value of the findings. Two other human case reports also suggest an association between RDX exposure and neurological effects.

Nervous system effects in experimental animals include a wide array of behavioral changes – irritability, hyperreactivity, tremors, and other signs that may be considered prodromal of convulsions - consistent with the induction of seizures by RDX exposure, and these actions have been observed in the majority of chronic, subchronic, and developmental studies examining oral exposure to RDX. There also is significant evidence from the scientific literature to indicate that RDX neurotoxicity results from interactions of the compound with the GABA_A receptor. These findings are consistent with the ability of RDX exposure to induce convulsions and related behaviors. While this evidence does not preclude the influence of other receptors as yet unstudied for RDX binding affinity, it does support involvement of the GABAergic pathway described above in the development of the

neurotoxicity. In contrast, evidence supporting a role for glutamate in the effects of RDX is very limited, and a basis for excessive glutamate stimulation in the assessment is weak, if not unfounded.

All hazards to the nervous system are adequately assessed, and there is no other more appropriate endpoint to address the spectrum of effects than convulsions, as these behaviors are observed in a significant proportion of the studies.

3a(ii) – Nervous system-specific toxicity values

The criteria utilized for the selection of studies reporting nervous system effects are scientifically sound and clearly described. By reviewing the scope of the search strategy and the process for identifying studies that report health effects and meet appropriate standards of quality for conduct, design, and reporting it is apparent to this reviewer that the most reliable scientific information has been accessed for this assessment.

The assessment has identified the data generated by Crouse *et al.* (2006) as a primary basis for judgments about the nervous system effects of RDX. This study and several others in the select group reporting effects on neurological health utilized gavage administration of RDX as opposed to a dietary route of administration. While dietary routes offer a more consistent ingestion and absorption of RDX and are perhaps better suited for chronic exposure studies, gavage procedures provide a more focused and intermittent administration of the compound. It is also true that there is less variability in the amount of the toxic agent delivered than for dietary intake that is dependent on the animal's feeding habits. As long as these characteristics of administration are understood and accounted for, there is no reason to exclude the work using gavage routes of exposure. Moreover, the Crouse *et al.* (2006) study produced perhaps the best RDX dose-response data available.

The most commonly reported nervous system effect of RDX is the induction of convulsions despite their character as severe functional endpoints. While many of these studies noted seizures as an observation, most of them did not track and report incidence or frequency or less severe behavioral signs that would support better analysis of this toxicity. The assessment presents the rationale for ascribing value to the Crouse *et al.* (2006) findings in a clear and logical manner. The potential relationship of convulsions to the mortality observed is also considered in the assessment, resulting in the conclusion from human and animal data that these two outcomes are not linked. The judgments in the assessment that led to these conclusions are appropriately described.

3a(iii) – Points of departure for nervous system endpoints

The most commonly reported nervous system effect of RDX is the induction of convulsions despite their character as severe functional endpoints. While many of these studies noted seizures as an observation, most of them did not track and report incidence or frequency or less severe behavioral signs that would support better analysis of this toxicity. The judgments in the assessment that led to this conclusion are scientifically supported and clearly described.

The calculation of PODs and HEDs for the selected studies are scientifically supported and clearly described. The severity of convulsions as an exposure outcome reasonably justifies the use of a benchmark response level of 1% extra risk. This value should be conservatively established, as the data on convulsions induced by RDX has limitations, particularly with respect to milder signs of enhanced neuronal activity that may precede seizures. Calculation of the lower bound on the benchmark dose for convulsions is appropriate and consistent with the EPA's guidance.

3a(v) – Nervous system-specific reference dose

The nervous system-specific reference dose for RDX is scientifically supported, conservatively established, and clearly characterized. The reference dose for the nervous system is very close to the value for the kidney/urogenital system, lending additional credence to both values. The nervous system dose is about an order of magnitude lower than that for the male reproductive system, but merits the highest confidence level of the three values and was also selected as the overall reference dose. The assessment considers the confidence levels for the non-nervous system doses to be low.

4a – Oral reference dose for effects other than cancer

The selection of the overall reference dose for RDX based on the nervous system effects described in Crouse *et al.* (2006) is scientifically sound, clearly described, and conservatively determined. This conclusion also takes into consideration mortality as well as the lower organ system-specific reference dose reported by Cholakakis *et al.* (1980) – the link of convulsions and mortality is weak, if not non-existent, and the study by Cholakakis *et al.* (1980) has greater limitations than that of Crouse *et al.* (2006).

Specifically, Cholakakis *et al.* (1980) was designed as a developmental toxicity study with only routine monitoring of clinical signs. This study used only three dose groups with order of magnitude spacing, resulting in a less reliable characterization of the dose-response curve for convulsions than by Crouse *et al.* (2006). The Cholakakis *et al.* (1980) study was also of much shorter duration and thus is less suitable for extrapolation to chronic exposure.

Executive summary

The executive summary presents a lucid and succinct description of the major conclusions of the assessment. This includes a compilation of the neurological signs, an explanation of the basis for the overall reference dose, a justification for exclusion of inhalation data, and a brief description of mechanistic evidence.

Dr. Melanie Marty

Charge Question 1. Literature search/study selection and evaluation. (Marty, Cobb) The section on *Literature Search Strategy/ Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations including exclusion criteria, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search strategy and study selection including inclusion and exclusion criteria, is fairly well-described and documented. EPA appropriately cast a wide net to retrieve all pertinent studies for the evaluation of health effects associated with RDX exposure. They searched PubMed, Toxline, Toxcenter, Toxic Substances Control Act Test Submissions (TSCATS), and the Defense Technical Information Center (DTIC) database, a central online repository of defense-related scientific and technical information within the Department of Defense. Studies were then screened to find those relevant to assessing the adverse health effects of exposure and developing a dose-response assessment. Citations in review articles and citations within original articles were also obtained and screened for additional pertinent information.

Figure LS-1 and Table LS-1 provide a summary of the general inclusion and exclusion criteria for studies that were kept for further evaluation of potential health effects of RDX. EPA used exclusion criteria such as excluding citations that were abstract only, on treatment and mitigation of environmental contamination with RDX, citations on laboratory methods, and those on the physical-chemical properties including explosivity. These were appropriate exclusion criteria, in this reviewer's opinion. These exclusion criteria resulted in the exclusion of over 900 references from further evaluation. Figure LS-1 could be clearer and better coordinated with the inclusion and exclusion criteria laid out in Table LS-1.

Table LS-1 indicates that studies on "ecological species" and non-mammalian species were also excluded. This seems to contradict statements (page xxix, lines 13-16) indicating that studies on nonmammalian species and ecosystem effects were considered as sources of information for the health effects assessment. Some clarification is in order. I note also that the exclusion of nonmammalian species may not be appropriate in light of the use of animals such as zebrafish in medium throughput assays to evaluate potential health risk to humans. Although there may be no studies of RDX in medium and high throughput assays, in the future these types of assays may provide at a minimum some mechanistic information for chemicals that could be used in health effects assessments.

Inclusion criteria in Table LS-1 were related to whether a citation was a source of health effects data pertinent to assessing the risk to humans (e.g., studies of health outcomes in RDX exposed humans or standard mammalian models by either the oral or inhalation route). Sources of mechanistic and

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toxicokinetic data were also included. Secondary references and other sources that described ecosystem effects, exposure levels, dealt with mixtures or were reviews or risk assessments and regulatory documents were excluded from study evaluation. However, EPA indicates that secondary references containing health effects data, and citations on non-mammalian toxicity were kept for consideration in the assessment. The description of what was done with secondary references could be clearer in Figure LS-1 and Table LS-2.

EPA provides details of the search in Appendix B, including search terms, and the number of hits per search term sequence per data base searched. They also tabulate the number of citations added to the database from their forward and backward web of science search of specific citations. Thus, the Agency has been very transparent in its process of identifying studies for evaluation.

EPA's evaluation of studies is fairly well-described and summarized in Table LS-3. The Agency used standard criteria and questions to evaluate study quality and utility that are described in several EPA guidance documents (cited in the assessment). Studies were evaluated considering the experimental design and conduct, issues around exposure to RDX, endpoints evaluated and presentation of results. EPA describes generally the issues they considered in evaluating the utility of both human and animal studies to inform both hazard identification and dose-response assessment.

EPA excluded 4 studies on health effects and described the reason for excluding these in Table LS-2. Similarly, EPA describes some of the important limitations in experimental animal studies in table LS-5. Overall, the description of EPA's study evaluation is clear, although the terminology is somewhat inconsistent (e.g., methodological features in Table LS-4 don't quite match the subheadings where these are described later in the section). Some details on strengths and limitations of specific studies chosen for further evaluation are provided in subsequent sections describing hazard identification and dose-response assessment for specific organ systems.

One additional comment relates to supporting evidence around sensitive subpopulations. Although there are not adequate studies on developmental neurotoxicity, there are some mechanistic studies implicating GABA agonist activity of RDX in the neurotoxicity observed in animals and humans. It would have been appropriate to search the literature for the role of GABA in brain development to inform EPA's assessment of sensitive subpopulations, to describe what is known to date and incorporate this information into considerations of sensitive subpopulations (e.g., infants, children, pregnant women and their fetus) as well as the uncertainty factors meant to account for variability in the human population.

Charge Question 3. a. iv. Nervous system. Uncertainty factors for nervous system endpoints (Section 2.1.3). (Marty, Stern) Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.¹

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EPA applied BMDS models to data from two gavage studies in rats (Crouse et al, 2006 and Cholakakis et al., 1980) to derive BMDL01 as a point of departure for effects on the nervous system following HED adjustment. A third data set (Martin and Hart, 1976) in monkeys was evaluated using the NOAEL approach. All three studies had data on incidence of convulsions, which EPA used as the nervous system toxicological endpoint for developing the RfD. EPA applied uncertainty factors to the HEDs.

Interspecies Uncertainty Factor (UF_A)

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the PODs to account for the toxicodynamic and residual toxicokinetic uncertainty not accounted for by the toxicokinetic modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive an HED.

Subchronic to Chronic Uncertainty Factor (UF_S)

EPA chose a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The agency partly rationalizes using a UF_S of 1 rather than a higher value by arguing that doses in the chronic feeding studies that induced convulsions were higher than those inducing convulsions in the shorter-term gavage studies. This does not seem to be a good argument. As EPA notes in the discussion of the studies, differences in RDX particle size in the dietary studies versus the gavage preparations have implications for absorption and internal dose, and may explain in part the differences in neurotoxic symptoms reported in the various dietary studies. The internal dose in the dietary studies with high particle size may have been significantly less than one might anticipate based on the administered dose. As EPA notes in Appendix C, pp. C-3-C-5, a number of studies have revealed that the preparation of the RDX media affects absorption. In particular, RDX administered orally as coarse particle preparation was much more slowly absorbed than as a fine particle preparation.

EPA also notes that the effect chosen as the endpoint (convulsions) is more related to dose than duration. This does argue for using a smaller UF_S than the standard 10 fold default factor, although a factor of 1 may be too low. The Agency could consider a UF_S of 2 or so to account for possible increased response with prolonged chronic exposure.

LOAEL to NOAEL Uncertainty Factor (UFL)

A UFL of 1 was applied because the BMDL was used as a point of departure. Thus, no extrapolation from a LOAEL to a NOAEL was needed. This is standard risk assessment practice and appropriate.

Database Deficiency Uncertainty Factor (UF_D)

The EPA applied a UF_D of 3 in developing an RfD based on neurotoxicity to help account for database deficiencies. This could be larger (e.g., 10) because there is limited information available to understand developmental neurotoxicity. There are reports indicating transplacental and lactational transfer of RDX in rodents; thus, there is exposure to the developing fetus and infant from maternal exposure. EPA notes that the two-generation reproductive and developmental toxicity study (Cholakos et al, 1980) did not report effects in the offspring at doses lower than maternally toxic doses. However, the study only looked at histopathology of the F2 pups at weaning. This study did not assess developmental neurotoxicity (DNT) in the offspring. The RDX assessment indicates that human studies did not demonstrate young age as a sensitive subpopulation, but there are no studies that actually evaluate this. There was one case report involving one child, but this one case study does not provide evidence regarding the influence of age at exposure on toxicity.

RDX interferes with neurotransmission by binding at the GABA_A receptor, and acting as an antagonist inhibiting GABAergic neurotransmission. GABA is a major inhibitory neurotransmitter in the adult brain. However, GABAergic systems play another role in vertebrate brain development acting as an excitatory neurotrophic factor contributing to processes involved in neurodevelopment (see for example Rivera, 1999; Kim et al., 2012). Lead (Pb), a potent developmental neurotoxicant, acts at least partly by inhibiting the GABAergic system during development in zebrafish (Wirbisky et al., 2014) and in mammals (reviewed in Neal and Guilarte, 2013). There is evidence that exposure of early postnatal rodent hippocampal slices to a GABA antagonist (bicuculline) reduces GABAergic neuroactivity, affects the regulation of GABAergic inhibitory synapses and increases their density in the hippocampus (Marty et al, 2000). The hippocampus is involved in seizure development in humans with epilepsy, so these results seem pertinent. There is evidence that drugs that act through the GABA_A receptor as GABA agonists can also cause neurodevelopmental disorders (see for example the review by Creeley, 2016). These lines of evidence point to potential window(s) of susceptibility in the developing brain for chemicals interfering with GABAergic systems. Given the often life-long sequelae of developmental neurotoxicity, a UF_D of 10 is warranted in this reviewer's opinion. Alternatively, the UF_{H-D} (toxicodynamic portion of the UF_H) can be increased to 10 to account for the uncertainty in toxicodynamics by age at exposure, in particular, in utero and infant/childhood exposure, given the window(s) of susceptibility during development of the brain to chemicals interfering with the GABAergic system. The total UF_H would then be 30.

Additional evidence prompting concern for developmental neurotoxicity is found in the section of the assessment on mechanism of RDX neurotoxicity. The assessment cites studies evaluating changes in gene expression including miRNA (p. 1-19, line 30-32), that report RDX upregulates 3 microRNAs that regulate brain-derived neurotrophic factor (BDNF). BDNF is important for brain development. As EPA notes, BDNF is a member of the neurotrophin family of growth factors, and promotes the survival and differentiation of existing and new neurons. Disrupting regulation of BDNF could result in developmental deficiencies in the brain if the perturbation of this pathway were strong enough. This provides additional indirect evidence to generate concern for potential developmental neurotoxicity of RDX.

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The overall UF for the neurotoxicity RfD is 100. The overall UF could, and in my view should, be higher. This reviewer recognizes that EPA chose to model a BMDL01 rather than a BMDL10 because of the severity of convulsions as an endpoint. That does not in and of itself account for the possibly widely different toxicodynamics by age at exposure. There is a strong argument that developmental neurotoxicity should be emphasized in evaluating the uncertainty factors applied, and that the total UF should be on the order of 300 rather than 100 for the nervous system RfD. Until there are modern developmental neurotoxicity studies on this compound, it is prudent to assume DNT is a possible outcome of RDX exposure. EPA does not discuss the role of GABAergic systems in neurodevelopment and the potential for interference with this system by RDX (or other compounds with similar molecular mechanisms) to induce developmental neurotoxicity, an omission that should be rectified.

The one reproductive toxicity study dosing pregnant dams that recorded incidence data for convulsions (Cholakis et al., 1980) provided a 5 fold lower POD and candidate RfD. This may indicate that pregnancy is a sensitive window for the adult for neurotoxicity. The Agency notes limitations in the data in terms of quantifying the dose-response relationship relative to the Crouse et al 2006 study chosen as the basis of the proposed RfD value. These limitations included a lower purity test compound, 14 days dosing rather than 90 days, and three widely spaced (order of magnitude) dose groupings versus 5 tightly spaced dose groupings in Crouse et al (2006), all of which impact the accuracy of a POD. This reviewer agrees that the RfD based on the Cholakis et al (1980) study is uncertain based on these data limitations, and that one cannot conclusively state that pregnant humans would be a sensitive subpopulation based on their findings. The observations of convulsions in the Cholakis study in pregnant dams at lower dose levels (2 mg/kg-d) than in the Crouse study (8 mg/kg-d) in nonpregnant female rats and resulting uncertainty about pregnancy as a sensitive time period for neurotoxicity of RDX could support use of a larger database deficiency factor. Pregnancy can change the toxicokinetics of agents, which may in part account for seemingly elevated susceptibility in the pregnant dams relative to nonpregnant animals in the other available studies. However, further study would be needed to confirm or refute this finding.

References cited in comment:

Kim et al., (2012) Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148(5):1051-1064

Rivera C et al (1999) The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397(6716):251-5.

Wirbisky et al. (2014) Novel dose-dependent alterations in excitatory GABA during embryonic development associated with lead (Pb) neurotoxicity. *Toxicol Letters* 229(1):1-8.

Neal and Guilarte (2013) Mechanism and lead and manganese neurotoxicity. *Toxicol Res (Camb)* 2(2):99-114.

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Marty S et al (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. J Neurosci 20(21):8087-8095.

Creeley (2016) From drug-induced developmental neural apoptosis to pediatric anesthetic neurotoxicity – where are we now? Brain Sci (6)32:

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population. Although a PBPK model was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose, EPA notes that not enough toxicokinetic data were available from human studies to model the differences among humans.

This reviewer agrees that the UF_H needs to account for both toxicodynamic and toxicokinetic variability among humans. EPA used the standard UF_H of 10, which is typically thought of composed of a half log for toxicokinetic differences and a half-log for toxicodynamic differences. Toxicokinetic differences among humans can be related to age, pregnancy, illness, medication use, other chemical exposures, and so on. In the absence of adequate toxicokinetic data to model the range of differences among humans, such differences must be accounted for by including a toxicokinetic component in the UF_H. The toxicodynamic portion of UF_H should cover differences in receptor-mediated response across humans. I note above in discussing the UF_D that the total uncertainty factor could be higher based on potential developmental neurotoxicity. Accounting for potential developmental neurotoxicity could also be achieved by increasing the toxicodynamic component of UF_H (e.g. increasing UF_{H-D} from 3 to 10 for a total UF_H of 30). The Agency should seriously consider doing one or the other to achieve a total UF of at least 300.

Charge Question 3. b. iv. Uncertainty factors for kidney and other urogenital system endpoints (Section 2.1.3) (English, Marty). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

EPA used suppurative prostatitis in a 2 year study in male rats (Levine et al., 1983) as a surrogate for the totality of observed adverse effects of RDX exposure on the kidney and urogenital system. EPA applied the BMDS models to the data from Levine et al using a 10% BMR. They then calculated HED based on three methods. Uncertainty factors were then applied to the BMDL10 HED to derive an RfD specifically for the kidney and urogenital system.

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population, which is standard risk assessment practice. Although a PBPK

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model was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose, EPA notes that not enough TK data were available to model the differences among humans. This reviewer agrees with not lowering the UF_H below 10 despite the PBPK modeling, which primarily describes the interspecies differences in toxicokinetics. Differences among humans can result from age, pregnancy, illness, medication use, and other chemical exposures. These must be accounted for by including a toxicokinetic component in the UF_H .

Interspecies Uncertainty Factor (UF_A)

An interspecies uncertainty factor, UF_A , of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the PODs to account for the remaining toxicodynamic and residual toxicokinetic uncertainty not accounted for in the TK modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive an HED.

Subchronic to Chronic Uncertainty Factor (UF_S)

EPA chose a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The Levine et al (1983) study was a chronic duration exposure study, and thus no extrapolation factor is needed. This is appropriate.

LOAEL to NOAEL Uncertainty Factor (UFL)

A UFL of 1 was applied because the BMDL was used as a point of departure. Thus, there is no need to extrapolate from a LOAEL to estimate a NOAEL. This is standard risk assessment practice and appropriate.

Database Deficiency Uncertainty Factor (UF_D)

The EPA applied a UF_D of 3 in developing an RfD for kidney and urogenital effects. The EPA notes that additional studies on neurotoxicity may provide a more sensitive endpoint to use as the basis of an RfD. Thus, the Agency applied a UFD of 3 across all POD, regardless of endpoint. Earlier comments by this reviewer (see charge question 3. b. iv.) indicated this could be increased to 10 due to the concern about developmental neurotoxicity.

Charge Question 3. c. iv. Uncertainty factors for reproductive system endpoints (Section 2.1.3) (Marty, Stern)). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

EPA used the data on testicular degeneration in mice from a 2 year dietary study (Lish et al. 1984). The Agency applied BMDS models to the incidence data to derive a BMDL for a 10% BMR. EPA used three methods to derive an HED from the mouse POD. The EPA notes that the toxicokinetic data available for the mouse are not as robust as for the rat, and thus they did not have confidence in the PBPK modeling to account for interspecies toxicokinetics. Rather, they chose the HED using the

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default extrapolation from animals to humans of scaling dose by $\frac{3}{4}$ power of body weight. After adjusting the mouse POD to an HED with this scaling, they applied uncertainty factors to derive an RfD for male reproductive toxicity.

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population. Although PBPK modeling was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose for some studies, EPA notes that not enough human toxicokinetic data were available to model the differences among humans. This reviewer agrees with not lowering the UF_H below 10 despite the PBPK modeling, which primarily describes the interspecies differences in toxicokinetics. Differences among humans can result from age, pregnancy, illness, medication use, and other chemical exposures. These must be accounted for by including a toxicokinetic component in the UF_H.

Interspecies Uncertainty Factor (UF_A)

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the POD to account for the remaining toxicodynamic and residual toxicokinetic uncertainty not accounted for by scaling dose using $\frac{3}{4}$ power of body weight. This is standard EPA risk assessment practice.

Subchronic to Chronic Uncertainty Factor (UF_S)

EPA chose a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The Lish et al, 1984 study was a 2 year dietary feeding study, and thus no extrapolation factor is needed.

LOAEL to NOAEL Uncertainty Factor (UFL)

A UFL of 1 was applied because the BMDL was used as a point of departure. This is standard risk assessment practice and appropriate.

Database Deficiency Uncertainty Factor (UF_D)

p. 2-12 and 2-13. The EPA applied a UF_D of 3 in developing an RfD for kidney and urogenital effects. The EPA notes that additional studies on neurotoxicity may provide a more sensitive endpoint to use as the basis of an RfD. Thus, the Agency applied a UFD of 3 across all POD, regardless of endpoint. Earlier comments by this reviewer (see charge question 3. b. iv.) indicated this could be increased to 10 due to the concern about developmental neurotoxicity.

Charge Question 3. c. v. Reproductive system-specific reference dose (*Marty, Meistrich*) (Section 2.1.4). Is the organ/system- specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

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The RfD for reproductive effects based on testicular degeneration is scientifically supported and clearly characterized. This effect was reported in one 2 year study in mice where exposure was through the diet (Lish et al., 1984), and also in a 2 year dietary study in rats (Levine et al., 1983) but only at an interim sacrifice. The Agency provided the BMDS analysis in Appendix D, and described the rationale for deriving the HED and applying the uncertainty factors. This reviewer agrees that the effect of testicular degeneration is an important male reproductive effect, although not reported in most studies. This effect was the best to use for dose-response assessment. Other reproductive effects included changes in testicular absolute and relative weight, but these findings were inconsistent across studies. Effects on fertility were noted in the 2-generation reproductive study at the high dose, but the animals had decreased weight gain and increased mortality and thus it was difficult to attribute the reduction in fertility to a specific effect of RDX. In the one dominant lethal assay, decreased rates of pregnancy of untreated females when mated with high dose males (F0 males from the two-generation study) may have been associated with generalized toxicity in the treated males rather than a specific effect of RDX. Thus, overall, the RfD specifically for reproductive effects is the best that can be done with available data. Note that EPA appropriately rated this RfD as having low confidence (Table 2-4, and page 2-18) because it is based on just one mouse study with no available confirmatory mouse study.

Dr. Marvin Meistrich

3.c.i - **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

A thorough review of the reports (a few publications but mostly unpublished technical reports) is presented.

The conclusion of suggestive evidence of male reproductive effects is based on evidence of testicular degeneration in male mice by Lish et al. (1984). As described in 3.c.ii, there are several weaknesses with this evidence.

On the other hand, Cholakakis et al. (1980) shows a reduction (which I calculate are significant) in pregnancy rates at the high dose group when males were treated. I suggest adding a table such as the one suggested below.

Reference and study design	Results					
<i>Pregnancy rates</i>						
Cholakis et al. (1980)	Doses	0	5	16	50†	50†
Dominant Lethal Study (Only males treated)	Incidence Percent	85/88 97%	87/88 99%	79/88 ^a 90% ^a	60/76* 79%*	
F0 of 2-generation study Both parental rats treated	Incidence Percent	17/22 77%	21/22 95%	20/22 91%	11/22 ^b 50% ^b	11/17 65%

* Statistically significant ($p < 0.05$); reviewer's calculation

^a Suggestive of statistical significance ($p = 0.07$)

^b Suggestive of statistical significance ($p = 0.06$)

† Presentation of data from 2-generation study was unclear; 22 females tested; 17 females co-housed, uncertain as to what should be the denominator

Examining the pregnancy data from the 2-generation study (Cholakakis et al, 1980), it is apparent that the loss of statistical significance of the pregnancies in the 50 mg/kg-day group may be a result of the incidence of pregnancies in the control group being lower than those in the 5 and 16 mg/kg-day groups. Although the conclusions in the draft that these effects may be due to the general toxicity of RDX or a treatment-related decrease in well-being of the males in the high-dose group, they may very well be indicative of reproductive toxicity towards the male and/or the female.

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In addition, the effects of RDX on fetal and offspring survival, and offspring development (Cholakis et al, 1980) (Table 1-10, Page 1-46) should be given more emphasis. The survival at weaning (8% in F1 of the 2-generation study and 0% in F2) is highly significantly different from the control values (87% and 79% respectively). Even though there is some toxicity at this dose (18% mortality, ~10% reduced body weights, ~14% reduced food consumption), the effects on fetal and offspring survival are huge and very important, and deserving of more consideration.

3.c.ii - **Reproductive system-specific toxicity values** (Section 2.1.1). Is the selection of the [Lish et al. \(1984\)](#) study that describes male reproductive system effects scientifically supported and clearly described?

The Lish report provides very weak suggestive evidence that the highest doses produce testicular degeneration by histological assessment. There are numerous weaknesses in the results and the presentation.

In the Technical Report and the draft Toxicological Review of RDX (Page 1-39, line 30) reference is made to comparison with historic controls. It is impossible to make such a comparison because it was done by different investigators (Ward et al., 1979) no quantitative level of what constitutes testicular degeneration is presented. The comparisons with historic controls should be deleted. There are problems in 2-year chronic studies of reproduction in mice and rats. At 2 years of age there is spontaneous deterioration in spermatogenesis (Zhang et al. 2006; Beattie et al. 2015). Significant effects may be due to combined effects of chemical and aging and not a result of the prolonged treatment. This caveat should be mentioned for all the 2-year chronic studies. No treatment induced testicular defects were reported by Lish et al. (1984) for the 6-month or 1-year tissue harvest, indicating that these defects were not present, although fewer mice were analyzed at these times.

There are some questions about the statistical significance of the numbers. Lish did not claim that the incidence of testicular differentiation was not significantly increased from concurrent or historical controls. However, by a simple Fisher exact test, I found that the incidences of degeneration at 100/175 mg/kg-day (3/27) and at 35 mg/kg-day (6/59) were both significantly different from control at $p = 0.025$ and $p = 0.011$, respectively. I suggest that the significance of these results be re-examined with a proper test. If they are not statistically significant, I do not think they should be used for calculation of a Reference Dose.

The suggestion that there was testicular degeneration was weakened by the failure to find any significant decreases in testis weights. Testis weight were only 2% lower than control in the 35 mg/kg-day group and 6% lower than control in the 100/175 mg/kg-day group. This cannot be significant since there mice treated with the lowest 1.5 mg/kg-day also showed a 6% lower testis weight than control. One would certainly expect the rats that showed degeneration to have markedly reduced testis weights, but such an analysis was not performed. Furthermore, the failure of another study to observe any significant decreases in testis weights or histological lesions in a subchronic 13-week study at doses up to 160 and 320 mg/kg-day with the same mouse strain (Cholakis et al. 1980), further weakens the support for effect indicated in the Lish et al. (1980) study. Although the RDX

used by Cholakis et al. was of larger particle size than that used by Lish, they extended their study to higher doses at which, even though the absorption of the larger particles was not as efficient as with the smaller particles, it was sufficient to induce animal toxicity. Also since there is no evidence of bioaccumulation of RDX, there subchronic study should have been sufficient to show an effect but it did not.

There have been a number of studies in rats that have generally found no significant effect. In a 2-year chronic study, Hart et al. (1976) found no testicular degeneration or weight loss at doses up to 10 mg/kg-day. Similarly Levine found no effect of a dose of 8 mg/kg-day. However, but at 40 mg/kg-day there was a significant decline in testis weight (14%) and a significant increase in the percentage of testes showing germ cell degeneration at 12 months of treatment. Although the effect is significant, the fact that there was 40% mortality by this time may indicate that the testicular damage was secondary to general toxicity. Testis weights at 24 months were not meaningful since all rats of this strain developed Leydig cell hyperplasia/neoplasms by 2 years of age, and the histological results, claiming no germ cell degeneration, are questioned.

There have been 3 studies of testicular effects in 13-week subchronic studies. Two studies used administration by diet as had been done in all the above studies. Cholakis et al. (1980) found no changes in absolute testis weights or histopathological damage to testes at 28 or 40 mg/kg-day. Note that there was some effect of the higher dose on weight gain but no mortality. Similarly Levine et al. (1981a,b, 1990) found no changes in testis weight, degeneration of the seminiferous epithelium or mortality at 30 mg/kg-day. Even though there was 80% mortality at 100 mg/kg-day there were not testicular effects. Finally Couse et al. (2006) using gavage, which generally has greater potency than dietary administration, observed some mortality at doses between 8 and 15 mg/kg-day. Nevertheless there were no significant histological effects and the authors indicated no significant change in absolute testis weights (although by a t-test I found an 8% decrease at 15 mg/kg-day).

Thus, other studies in general fail to support the testicular toxicity observed by Lish et al. (1980). The only other study with a suggestive effect (Levine et al., 1983, results at 1 year), are also associated with animal toxicity leading to appreciable mortality.

3.c.v - Reproductive system-specific reference dose (Section 2.1.4). Is the organ/system- specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

Reference dose is calculated from BMDL for a small level testicular degeneration in mice. However, more severe effects on reproduction (pregnancies, fetal survival, offspring survival) are observed in rats and should be given more weight in determination of the Reference Dose for reproductive effects.

Specific comments on presentation of data on reproductive and developmental toxicity

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1. In Table 1-9, the relative testes weights should be deleted. Relative testis weight is affected by changes in body weights, which in our experience does not have effects on testis weights of adult animals. Absolute testis weights are a better measure of testicular toxicity of an agent. The relative testis weights just clutter up the table and add little information on the toxicity of RDX.
2. Table 1-9, Page 1-42: In the presentation of the data of Levine et al. (1983), the data on "SDMS" (spontaneous death or moribund sacrifice) rats should be deleted. Their significance is open to question and they aren't given much weight in the discussion.
3. Table 1-9, Page 1-44: The data on incidence of germ degeneration of Levine et al. (1981a,b, 1990) at 12 and 15 mg/kg-day should be deleted. These were observed on dead rats (all rats in these groups died). Incidentally the numbers are reversed: the value for 1/10 was for the 12 mg/kg-day dose and 1/9 was for 15 mg/kg-day.
4. The testis weight data from Cholakis et al. (1980) (Table 1-9, last entry on Page 1-43) on F2 weanlings does not belong in the male reproductive effect section. It is not indicative of direct effect on testis weight and there is no follow-up to determine whether or not adult testis weights will be affected. Rather it belongs in the developmental effects section (Table 1-10).

Additional References

Beattie MC; Adekola L; Papadopoulos V; Chen H; Zirkin BR. (2015). Leydig cell aging and hypogonadism [Review]. *Exp. Gerontol* 68: 87-91.

Zhang, X; Ebata, KT; Robaire B; Nagano M (2008). Aging and male germ line stem cells in mice. *Biol Reprod* 74: 119-124.

Dr. Marilyn Morris

1. Literature search/study selection and evaluation.

The literature review is comprehensive and exclusion criteria are appropriate.

2. Toxicokinetic modeling. In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans ([Sweeney et al., 2012a](#); [Sweeney et al., 2012b](#)).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

The changes to the PBPK model of Krishnan /Sweeney represent distinct improvements. Human metabolic rate constants were fitted from human data. Additionally, it is stated that in vitro data from rats and human metabolic studies were used, with scale-up to liver size based on microsomal protein. Oral absorption parameters were re-evaluated to take into consideration different dosage forms. The further EPA evaluation also performed validation of the PBPK model using independent rat data sets, and provided goodness of fit parameters.

Specific comments:

1. Despite these improvements in the model, the data in rats is not fitted well and shows substantial deviations especially at early time points. This may reflect significant deviations of the data from the model absorption parameters, and possibly in clearance. Further optimization may improve fitting. For absorption, this might include permeability data using human intestinal CaCo2 cells or investigating other intestinal models. For elimination, hepatic intrinsic clearance is preferred over a rate constant. From *in vitro* microsomal and S9 studies reported by Cao, data is provided that can be used to calculate metabolic intrinsic clearance. Cao demonstrated that the intrinsic metabolic clearance in a microsomal preparation was greater in humans than in rats and mice. However, concentration-dependent studies were not performed, so this publication does not provide support for the assumption of linear clearance.
2. Clearance terms instead of first order rate constants (dependent on elimination and the apparent volume of distribution) would be more informative in the model. In vitro (K_m/V_{max} or intrinsic metabolic clearance) or derivation of intrinsic clearance from fitted clearance obtained from in vivo data may be used.
3. Clearance is assumed to be entirely by metabolism, which is reasonable based on the accumulated data from many studies. Variability based on metabolic clearance could be built into the model (or by known variability in enzymes, such as CYP2B4 (a rabbit CYP

which is suggested as a CYP involved in RDX metabolism). This corresponds to CYP2B6 in humans.

4. The role is metabolites in toxicity is discussed in the document, and due to a lack of data this is not included in the model. This is appropriate in the model due to the lack of data and the fact that metabolites are not present to any extent in blood; however, there is no information on metabolites in brain and a potential role in neurotoxicity.
5. Tissue partitioning is mainly determined by in silico methods; more in vivo data would provide the justification for these values and decrease variability. The most important data would be partitioning into the brain since the major toxicity is neurological. Brain extracellular fluid concentration-effect relationships would be most informative and differences in binding to GABA_A between rodents and humans could also be incorporated; this would drive the neurological effects. That being said, plasma/blood concentrations may be linearly related to brain concentrations, and may be used to drive toxicity, as proposed, based on limited correlations observed with brain and plasma data from animal studies and data from the child after poisoning (CSF data).
6. Protein binding of RDX is not mentioned in the document: it is the free concentration that would diffuse across the BBB in the absence of any active uptake processes and will be metabolized by the liver. This could lead to differences in brain/blood ratios in humans, and may be helpful in allometric scale-up of clearance.
7. Use of Simcyp or Gastroplus which both have PBPK modules could facilitate modeling.

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

Since we are focusing on chronic toxicity, AUC is more appropriate. Additionally, peak concentrations are not predicted well from the PBPK models.

Only the dose metric for seizures/convulsions provides reasonable certainty. That for cancer endpoints does not.

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2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation if applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of different factor instead?

There are a number of uncertainties, including clearance, brain concentrations, protein binding, susceptibility to seizures, affinity for binding to GABAA receptors, oral absorption parameters, and metabolite contribution to toxicity which limit certainty. Overall, until we have a better understanding of the variability of PK, PD, and relevant concentration-effect relationships, the uncertainty factor needs to be large.

3. Hazard identification and dose-response assessment.

3a. Nervous system effects

- **Nervous system hazard** (Sections 1.2.1, 1.3.1). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

There doesn't appear to be an appropriate endpoint to address the spectrum of neurological effects, at this time. EEG and memory evaluations may provide earlier clinical endpoints.

- **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C. and in the context of specific hazards in the toxicological review), is it appropriate to consider the [Crouse et al. \(2006\)](#) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

Gavage and dietary administration will result in differences in Cmax and possibly in AUC (extent of absorption). Results from gavage studies are more reliable with

respect to dose administration, since there is more variability in dietary consumption based on amounts of diet consumed, the influence of taste/smell of RDX on dietary intake, and effects of food on absorption. Particle size may influence bioavailability if aqueous solubility/dissolution is rate-limiting for absorption. Generally, gavage studies should be appropriate, except at very high doses where the initial blood concentrations of RDX may be very high resulting in toxicity. This may be due to a rapid rate of absorption and possibly saturation of first pass metabolism.

- **Points of departure for nervous system endpoints** (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described?

The calculations of HEDs are scientifically supported and clearly described.

3b. Kidney and other urogenital system effects

Points of departure for kidney and other urogenital system endpoints (Section 2.1.2).

Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

The scientific data is variable with respect to toxicity involving the kidney and urogenital effects. However, the HED for neurological effects appears appropriate for kidney/urogenital effects based on scientific literature.

3c. Developmental and reproductive system effects

Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

There is little evidence for reproductive toxicity.

3e. Cancer

- (i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

I agree that the evidence is only suggestive.

- (ii) **Cancer-specific toxicity values**

- (iii) **Points of departure for cancer endpoints**

- 4. **Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>) in the following analyses.

4b. Inhalation reference concentration for effects other than cancer (Section 2.2).

The data does not support an inhalation reference concentration.

4d. **Inhalation unit risk for cancer** (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of a inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

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The available data does not support an inhalation unit risk.

Dr. Victoria Persky

3a. Nervous system effects

(i) **Nervous system hazard** (Sections 1.2.1, 1.3.1). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

Answer: This reviewer agrees with the overall assessment that nervous system toxicity is a human hazard of RDX exposure. The strongest data comes from controlled animal studies (Crouse 2006, Lish 1984, Levine 1983), with seizures and behavior changes, such as hyperactivity and fighting) apparent at the highest levels of exposure. Human studies are generally supportive, with the strongest evidence for convulsions coming from studies of acute exposures (Testud 1996, Hollander 1969, Merrill 1968 Barsotti, Stone 1969). The evidence presented for RDX possibly acting through GABA receptors is intriguing and supports the conclusion that RDX is toxic to the nervous system. There is limited data on other nervous system endpoints, with one study in humans showing lower levels of memory retention (Ma and Li (1993). Additional studies addressing cognitive and behavioral effects of RDX would assist in assessing other endpoints less severe than convulsions. Although there is data from existing animal studies showing changes in behavior, the data is probably not sufficiently robust to evaluate dose response and animal data on cognitive changes is lacking. Therefore, given these limitations, there are not endpoints in existing studies to address the complete spectrum of effects.

3b. Kidney and other urogenital system effects

(i) **Kidney and other urogenital system hazard** (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

Answer: This reviewer agrees with the conclusion that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. The transient changes in BUN and urine output after acute exposure in humans suggest that renal long term effects are possible but studies of long term exposure are few with numbers too small for conclusions. Animal data is more robust, but results vary, with some showing papillary necrosis, bladder distention and suppurative prostatitis, as well as changes in measures such as BUN and uric acid, but the results vary among studies and species. There is evidence in the animal studies that effects on the kidney contribute to mortality in the animals. The possibility that RDX may act through GABA receptors on the urogenital system indirectly supports potential toxicity. Arguments around whether suppurative prostatitis is the appropriate endpoint are described in the document. Reviewers at the Washington meeting elaborated on the limitations in using prostatitis as the endpoint, in part because of lack of precision in the diagnosis. Relying on more definitive endpoints, given the current evidence without clear dose response data could be problematic.

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3c. Developmental and reproductive system effects

(i) **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

Answer: This reviewer agrees with the assessment that there is suggestive evidence of reproductive effects but lack of conclusions concerning developmental effects of RDX exposure. There appears to be limited data on effects of RDX on reproduction and development, with no human data available. There is some evidence of testicular degeneration that is suggestive of male reproductive effects. Changes in fertility occurred only at the highest doses where this effect could be attributed to overall decline in health of the animals. Similarly the differences in growth and survival at high doses could be attributed to general poor health of the animals.

3d. **Other noncancer hazards** (Sections 1.2.4, 1.2.6, 1.3.1). The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision. Are other non-cancer hazard adequately described?

Answer: This reviewer agrees with the overall lack of conclusions regarding other non-cancer effects. There is suggestive evidence of transient liver effects after acute toxicity in human studies, although the increases in liver enzymes in most cases reversed and the animal data is inconsistent. The lack of consistency in animal histology and the possibility of contamination in some studies with HMX supports the report's caution in interpreting the results. Expanded discussion of specific endpoints, other than liver, would enhance the document. The inconsistencies in lipid and ocular results do not allow for inferences to be drawn.

3e. Cancer

(i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

Answer: This reviewer agrees with the conclusion that there is suggestive evidence of carcinogenic potential for RDX. This is supported by the animal studies showing increases in benign and malignant liver tumors as well as lung cancers. The results, however, are not consistent among studies, species and gender. The discussion concerning potential mechanisms is thorough. Apparently there is no evidence that RDX itself is mutagenic. While there is some evidence that the N-nitroso metabolites might be mutagenic evidence that these metabolites are operative here is lacking.

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4b. Inhalation reference concentration for effects other than cancer (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

Answer: This reviewer agrees with the assessment that available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis.

Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment

Answer: This reviewer feels that the executive summary is clear and adequately presents the major conclusions

Dr. Issac Pessah

1. **Literature search/study selection and evaluation.** The section on *Literature Search Strategy/Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations **including exclusion criteria**, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search that was conducted used a systematic, and unbiased set of search terms with clearly defined inclusion and exclusion criteria to assess primary studies reporting on the primary toxicological outcomes of RDX to animals and humans, including clinical case reports. Several strength of the literature search include its comprehensiveness and the use of PECO statements for delineation of differences in study designs with respect to population, exposure parameters (such as route, timing and purity of RDX), and relevant comparisons were made across doses and routes. In general, potential hazard outcomes were clearly defined and appropriate.

With respect to nervous system hazards for RDX, there were sufficient data to link RDX exposures to convulsions but insufficient information on which guide conclusions about more subtle neurological and behavioral outcomes from sub-convulsive exposures. Moreover, there was a lack of definitive data on the potential influences on neurological and behavioral outcomes after RDX-triggered seizure resolved. Considering that the AOP for RDX is likely due to direct interference with GABA-A receptor functions in the CNS, inclusions of relevant studies on compounds having the same mode of action may have provided additional important clues of the potential hazard of RDX in promoting more subtle neurological (cognitive), behavioral (anxiety) and/or developmental neurotoxicity.

3a. Nervous system effects

- (i) **Nervous system hazard.** The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. **Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?**

Human Studies

There is consistent evidence from more than 20 clinical case reports that exposure to RDX is associated with adverse neurological outcomes, particularly an association with convulsions. Never the less, there are many and varied limitations to concluding about the hazards of RDX solely based on such case reports. There is only one cross-sectional study that provides a snapshot of the potential neurotoxicity associated with pulmonary exposure to RDX. Ma and Li (1993) presented results from a number of neurobehavioral functioning and memory tests from a study of workers exposed in a Chinese RDX plant at a single point in time. The results indicated significant neurobehavioral and memory deficits were associated with RDX exposure measured in air. However, this study has several significant limitations that should limit conclusions about

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RDX hazard solely based on its findings. Of greatest concern was the omission of exposure levels in the “non-exposed” group, no attempt to control for confounders (non-occupational exposures, lifestyle confounders, influences of co-morbidity factors), and the lack of a rationale for subdividing the exposed cohort. Never the less, the outcomes on Composite Memory Retention Quotient and Composite Block Design score were greater >15 points and ≥ 2 sec lower ($p < 0.01$) than the control group, respectively. Statistical analyses seem appropriate but 95% CIs would have been helpful since the magnitude of functional impairments across groups is within the High average [110-119] and Average [90-109] range, measures typically associated with a 15% SD.

Animal Studies

Several studies with rodents using oral gavage and dietary exposure models over the acute, sub-chronic and chronic timeframes have consistently identified a broad range of neurological impairments ranging in severity. Convulsive (seizure) activity is a common in most, but not all, studies. In addition to seizure activity, several of these studies identified “less severe” neurological and behavioral impairments that may be consistent with the sparser literature on human exposures. An important observation stemming from some of the animal studies is that RDX appears to sensitize animals exposed to lower doses to subsequent seizurogenic stimuli, including electrogenic, audiogenic, and chemical kindling. Another potentially important experimental finding is that exposure dose appears to be a more important predictor of adverse neurologic outcomes than is duration of treatment.

Mechanistic Studies

The neurotoxicity profile of RDX is consistent with that of a centrally acting excitotoxicant. There is ample direct evidence of a direct interaction of RDX with GABA-A receptors (GABR) in the mammalian CNS. RDX blocks GABA activated Cl^- currents and the inhibitory postsynaptic potentials (iPSPs) that form critical inhibitory networks throughout the brain. The available data also suggest that the limbic system, including the amygdala, is especially sensitive targets of RDX. Surprisingly, the potency of RDX as a GABR blocker is relatively low. By comparison picrotoxin (PTX) has >100 -fold lower K_i (100x more potent) than RDX at binding to GABR (K_i 0.2 vs 21 μM). The lower potency of RDX extends to the concentrations needed to inhibit Cl^- currents in whole cell voltage clamp experiments and iPSC events, which typically require $\geq 10\mu\text{M}$. Also relevant to RDX mechanism and its potential importance to long-term behavioral toxicity is the observation that the inhibitory actions of RDX on seizure-like neuronal discharges measured in the basolateral nucleus of amygdala.

Preliminary Conclusions

Despite the limitations of the Ma and Li (1993) study, considered with the sum of evidence from clinical case reports, results from experimental animals, and mechanistic studies on RDX, there is necessary and sufficient evidence to support the conclusion that nervous system toxicity is a human hazard of RDX exposure. With respect to whether all hazards to the nervous system have been adequately assessed, the measure of abnormal electrographic activity or seizure-like activity in specific brain regions may be a more sensitive indicator of neurotoxicity and the potential of RDX to elicit more subtle neurological impairments such as cognitive deficits and/or behavioral

abnormalities.

(ii) **Nervous system-specific toxicity values.** Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the [Crouse et al. \(2006\)](#) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

The selection of studies reporting nervous system effects are scientifically supported, clearly described, and sufficient to draw conclusions about the potential hazards associated with exposures to RDX. The differences in toxicokinetics of RDX exposure by gavage vs. dietary administration is clear, and must be accounted for when predicting risk. Based on the state of the science, it appears that the gavage route for RDX results in high peak blood and brain levels than the dietary route. Also the rate of rise in blood and brain levels is faster with gavage. Since it is the dose rather than the duration of exposure that is more predictive of neurological outcomes, at least from animal studies, it is appropriate to consider the dose-response data reported in Crouse et al. (2006) study as a relevant model that should be protective of dietary and dermal exposure paradigms. However, it is not likely that the results from Crouse et al 2006 would be protective of pulmonary exposures which may lead to faster and higher blood/brain levels at equal dose. There remain several unanswered questions (uncertainties) with regard to the dose-response relationships leading from seizure to convulsive activity and lethality. Based on the available data, death may occur without seizure or convulsions detected, although this may simply be due to the lack of adequate (sufficiently frequent) observations by the experimenters. However, based on the current state of the science (including the epilepsy literature), death is not a necessary outcome of seizures or convulsions, and is driven by abnormal electrographic patterns in the brain. It is therefore reasonable to conclude that convulsion as characterized in the report is a reasonable severe endpoint for human risk assessment.

(iii) **Points of departure for nervous system endpoints. Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described?** Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

The selection of convulsions as the endpoint to represent the most severe endpoint for nervous system hazard is scientifically supported and clearly described in the document. Several, more subtle cognitive and behavioral neurological impairments are not considered and integrated into the report per se, although there is strong evidence from several clinical reports, the cross-sectional study of Ma and Li (1993), and a number of rodent studies that they can occur without evidence of convulsion. This raises the question of whether calculations of PODs and HEDs,

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although scientifically supported and clearly described in the document, are in fact protective of more neurological impairments in the absence of convulsion. Never the less, several factors mitigate this concern: (1) the intrinsic potency of RDX for binding GABR is >100-fold lower than that of a comparable ligand (PTX) with the same AOP; (2) the acute dose required for eliciting convulsions is >100-fold higher than that required for PTX; (3) the indication that dose rather than exposure duration is a more important indicator of adverse outcomes, and (4) evidence from animal studies of other seizurogenic compounds with similar AOP that indicate the LOELs for triggering abnormal electrographic patterns and those that produce convulsions are within a factor of 2-3 fold dose range.

Based on the sum of the data available, it is concluded that the severity of convulsions warrant the use of a benchmark response level of 1% extra risk. Base on the available science, use of the BMDL for convulsion is considered appropriate and consistent with the EPA's Benchmark Dose Guidance.

(iv) **Uncertainty factors for nervous system endpoints.** Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.2

The POD for neurological effects is based on the most detailed D-R data available. The gavage route of exposure should be protective of the more human-relevant dietary route because the former yields more rapid distribution and higher blood levels than the latter. However, whether the uncertainty factors will be protective of pulmonary exposure to fine RDX dust remains uncertain. Another uncertainty is whether differences in human susceptibility ($UF_H = 10$) is defensible given the growing number of highly penetrant gene variants associated with genes that encode human GABR subunits that confer and/or enhance susceptibility to not only seizure disorders but also behavioral impairments.

(v) **Nervous system-specific reference dose** (Section 2.1.4). Is the organ/system- specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

Based on the available data, the nervous system RfD is supported by the available scientific literature and clearly presented.

Executive Summary

The executive summary is for neurological effects of RDX is presented in a clear and concise manner. It accurately summarizes the strengths and weaknesses of all the reliable data.

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Dr. Kenneth Portier

1. Literature search/study selection and evaluation. All Members

- Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described.

As I reviewed this section I was looking for transparency in description of search and evaluation procedures, clear description of available data and assessment of study quality. Using these criteria I found the literature search, study selection and evaluation section excellent. I have only one item to discuss.

One exclusion criteria does not appear to be well described or discussed. In Table LS-1 second row, third bullet in the Exclusion criteria column: "Exposure via injection (e.g. intravenous [i.v.])". I understand why data/findings from this type of study is not useful in informing environmental exposures since intravenous injection is not likely to be a common or likely route of exposure. There may be situations where i.v. exposure study findings could be useful and informative in other parts of the assessment, for example when evaluating PBPK models. Does exclusion here mean these types of studies never appear in the report?

- Please identify additional peer-reviewed studies that the assessment should consider.

No additional studies to add.

2. Toxicokinetic modeling (Appendix C, Section C.1.5).

Questions 2a. Barton, Morris, Bruckner

- Are the conclusions reached based on EPA's evaluation of the models scientifically supported?
- Do the revised PBPK models adequately represent RDX toxicokinetics?
- Are the model assumptions and parameters clearly presented and scientifically supported?
- Are the uncertainties in the model appropriately considered and discussed?

Questions 2b. Barton, Morris, Bruckner

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- Is the choice of dose metric for each hazard sufficiently explained and appropriate?
- Is the decision to not use the mouse PBPK model to derive PODs for non-cancer or cancer endpoints due to uncertainties in the model and to uncertainties associated with selection of a dose metric for cancer endpoints, scientifically supported?

Questions 2c. (section 2.1.3) **Barton, Morris, Bruckner**

- Does the toxicokinetic modeling support the use of a value other than the uncertainty factor of 10 for human variation in the RfD used in the draft assessment.

3. Hazard identification and dose–response assessment.

3a. Nervous system effects

(i) Nervous system hazard (Sections 1.2.1, 1.3.1). Lasley, Miller Persky, (human studies), Pessah, Reddy

- Please comment on whether the available human, animal, and mechanistic studies support the conclusion that nervous system toxicity is a human hazard of RDX exposure.
- Are all of the hazards to the nervous system adequately discussed?
- Is convulsions the appropriate endpoint for addressing the spectrum of neurologic effects from RDX?

(ii) Nervous system-specific toxicity values (Section 2.1.1). Lasley, Miller Pessah, Reddy

- Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described.
- Is it appropriate to consider the [Crouse et al. \(2006\)](#) study, which used gavage administration, in establishing a nervous system-specific toxicity value, given the difference in toxicokinetics between gavage and dietary administration (see Appendix C, Section C.1)?
- Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

(iii) Points of departure for nervous system endpoints (Section 2.1.2). BMD Modeling –

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Hughes-Oliver, Portier, Stern; HED extrapolation using PBPK modeling – Barton, Morris

- Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described?

The argument for the selection of three studies; the two gavage studies, Crouse et al. (2006) and Cholakakis et al. (1980) and the one dietary study of Levine et al. (1983) for dose response analysis seems sound. The first sentence in Section 2.1.2 notes that “No biologically based dose-response models are available for RDX.” Can we imply for this that none is possible with available data/information or that none was attempted?

- Are the calculations of PODs for these studies scientifically supported and clearly described?

Yes. The method of analysis is described in sufficient detail to allow the reader, utilizing the data reported in Appendix D and publically available BMDS software, to duplicate the analysis.

- Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk?

In Section 7 of the preface we find “For human studies, an assessment may develop exposure–response models that reflect the structure of the available data (U.S. EPA, 2005aU.S. EPA, 2005aU.S. EPA, 2005aU.S. EPA, 2012, §3.2.1). For animal studies, EPA has developed a set of empirical (“curve-fitting”) models⁶ that can fit typical data sets (, §3.2.2). Such modeling yields a *point of departure*, defined as a dose near the lower end of the observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower confidence limits)(, §3.2.4), (, §2.2.1).”

On page 1-71 we are told that “Convulsions, considered a severe adverse effect, were selected as a consistent and sensitive endpoint representative of nervous system effects.” All further references to convulsions refer back to this statement, which does not argue that convulsions are a severe adverse effect but simply states it. A better discussion on severity can be found on page 2-12 in the bullets related to uncertainties in the database. Here we find “ In the available studies, “convulsion” can indicate a range of observable behaviors in response to altered brain activity, ranging from involuntary limb and facial twitches to tonic-clonic seizures in which

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animals exhibit a sustained (seconds to hours) and widespread loss of muscle control sometimes resulting in respiratory arrest and/or death. As there are studies where convulsions occur at the same dose as mortality, the convulsive activity in these studies is interpreted as severe." I think this discussion should be moved and expanded in Section 1.2.1

It can only be assumed that the argument on page 2-12 is the argument for severity of convulsions and hence the choice of a 1% benchmark response level in estimating the POD. But this same paragraph is a major component for the argument for setting an data uncertainty factor (UF_D) of 3 in converting the estimated POD to the Human Equivalent Dose (HED or Candidate value, see Table 2-3). It feels like one or the other should be used, but not both. On the other hand, the UF_D argument also includes uncertainties in the impact of RDX seizure-related behaviors on development. So maybe a factor of 3 to cover this uncertainty alone is acceptable.

There is also the issue of whether using a 1% BMD represents a "significant extrapolation" of the available data. (Note that page 2-6 states that "A BMR of 1% extra risk (ER) for convulsions was used to address the severity of this endpoint; the BMD and BMDL estimates for 5 and 10% ER for the selected model are provided in Appendix D (see Section D.1.2, Tables D-3 to D-7) for comparative purposes." These estimates are found in the footnotes of the tables indicated.)

For combined sex Crouse et al. (2006) data, the $BMD_{1\%}$ estimate (3.02) is quite close to the lowest dose (4.0) and does not represent a significant extrapolation. For the Cholakakis et al. (1980) data the $BMD_{1\%}$ estimate (0.179) is significantly below the lowest dose of 4. For the Crouse et al. (2006) gavage data, the $BMD_{1\%}$ estimate (2.56) is actually above the second lowest dose in the study (2). In almost all cases the $BMD_{10\%}$ estimates are within the range of non-zero doses in each study. In general one can conclude that use of the $BMD_{1\%}$ does not represent significant extrapolation.

- Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

The approach described in Appendix D, Section D.1.2 is consistent with EPA's Benchmark Dose Guidance (BDG) found in EPA (2012). A particularly nice feature of this presentation is the inclusion in the tables summarizing the model fits (Tables D-3 to D-7) of narrative laying out the basis for model selection.

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As shown in Tables D-3 to D-7, 12 models were fit to available animal data, with goodness of fit P-value and AIC estimates provided for each along with the estimated BMD_{1%} and BMDL_{1%}. In a couple of cases one model was excluded due to significant lack of fit. The remaining models had very similar fits as measured by the AIC. The final selected models seem reasonable as can be seen in Figures D-1 to D-5.

For very little additional effort, model predictions for all 12 fitted models could have been displayed in the figures, providing the reader an indication of the heterogeneity of model forms fit, and further supporting the choice of the specific model used.

Note that in all three modeling exercises, the estimated AICs of acceptable models are practically identical requiring further justification to select the one model used. While not part of current EPA Benchmark Dose Guidance, benchmark model averaging (Wheeler and Bailer (2007) is being used to derive a BMD and benchmark dose lower confidence limit (BMDL) estimates having better statistical properties than any one particular model. Essentially, model averaging uses a weighted average of model predictions, where the weights come from the Akaike Information Criterion (AIC) with the result that better fitting models have greater influence on the average curve. The BMDL_{1%} is estimated using the appropriate percentile of the BMD_{1%} from the bootstrapped samples. This approach addresses model uncertainty directly rather than forcing analysts to “pick a winner” among models having fairly similar fits but very different parameterization and extrapolation characteristics. Model averaging has been shown to outperform selection of the single best fit model in terms of bias and coverage of the one-sided confidence interval for the BMD_{1%} used to calculate the BMDL (Wheeler and Bailer, 2007). It also produces more stable estimates than individual models (see, for example Yuan and Ghosh, 2009). Finally, EPA has publically available software, the MADr-BMD, that extends the functionality of the current BMDS model fitting package and has been extensively tested and been peer reviewed (Wheeler and Bailer, 2008). Model averaging makes two assumptions that are typically met in these kinds of analyses. The underlying models being averaged must be monotonic in character and the overall analysis must use enough models to capture a wide range of potential dose-response relationships. The 11 models adequately fit to these data represent a wide range of potential relationships. Wheeler and Bailer (2007) recommend excluding from the set of models averaged any that don't match the mechanistic assumptions of the toxicant or for which the BDDS package has trouble in estimating model parameters. With this in mind, the 12 models from the BMDS package should be compared to the expectations from the PBPK model results to determine if any should be excluded.

1. US EPA. (2012). Benchmark Dose Technical Guidance, Risk Assessment Forum, Washington, DC. Publication #EPA/100/R-12/001.

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2. Wheeler, MW and Bailer, AJ. (2007). Properties of Model-Averaged BMDLs: A Study of Model Averaging in Dichotomous Response Risk Estimation. Risk Anal. 27: 659-670. <http://dx.doi.org/10.1111/j.1539-6924.2007.00920.x>.
 3. Wheeler, MW and Bailer, AJ. (2008). Model Averaging Software for Dichotomous Dose Response Risk Estimation. J Stat Software. 26. <http://dx.doi.org/10.18637/jss.v026.i05>.
 4. Yuan, Z and Ghosh, D. (2009). An improved model averaging scheme for logistic regression. Journal of Multivariate Analysis. 100: 1670-1681.
- *Are the calculations of HEDs for these studies scientifically supported and clearly described?*

(iv) **Uncertainty factors for nervous system endpoints (Section 2.1.3).** English, Marty, Stern

- Is the application of uncertainty factors to these PODs scientifically supported and clearly described?
- Please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.

See my paragraph 3 above in the discussion about the justification for the BMD_{1%}.

(v) **Nervous system-specific reference dose (Section 2.1.4).** Lasley, Miller Pessah, Reddy

- Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

3b. Kidney and other urogenital system effects

(i) **Kidney and other urogenital system hazard (Sections 1.2.2, 1.3.1).** Borland, Lash, Persky (human studies), Rosol

- Please comment on whether the available human, animal, and mechanistic studies support the conclusion that kidney and other urogenital system toxicity is a potential human hazard of

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RDX exposure.

- Are all of the hazards to the kidney and other urogenital system adequately discussed?
- Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

(ii) **Kidney and other urogenital system-specific toxicity values (Section 2.1.1).** Bosland, Lash, Rosol

- Is the selection of the [Levine et al. \(1983\)](#) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

(iii) **Points of departure for kidney and other urogenital system endpoints (Section 2.1.2).** BMD Modeling – Hughes-Oliver, Portier, Stern; HED extrapolation using PBPK modeling – Barton, Morris

- Is the calculation of a POD for this study scientifically supported and clearly described?
- Are the calculations of HEDs for these studies scientifically supported and clearly described?

A BMR of 10% was used for estimating the POD. The only justification for using this level is given on page 2-6, "A BMR of 10% ER was applied under the assumption that it represents a minimally biologically significant level of change." This represents only minimal description of the POD choice and is only scientifically justified if the discussion under section 3b(i) finds this outcome acceptable as the effect endpoint.

Estimation of the POD follows the approach described in Appendix D, Section D.1.2 and is consistent with EPA's Benchmark Dose Guidance (BDG) found in EPA (2012). A particularly nice feature of this presentation is the inclusion in Table D-9 summarizing the model fit of narrative laying out the basis for model selection. Note that the final selection criteria is that the model produces the lowest BMDL_{10%} estimate. This should be a conservative value.

As shown in Tables D-9, 10 models were fit to available animal data, with all models having acceptable goodness of fit and very similar AIC estimates. The final selected model, the Log-Probit, seems reasonable as can be seen in Figure D-7. The estimated BMD_{10%} (1.67) is within the range of study doses so there is no issue of inappropriate extrapolation.

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My comments presented in the section on nervous system endpoint POD estimation, relating to plotting all model fits in Figure D-7 and the potential use of model averaging also hold for this endpoint. Note that the fitted model displayed in Figure D-7 is monotonic but concave in form suggesting a plateauing of response. It is not clear that all 9 models can adequately accommodate this kind of response (concave form), hence the desire to see all model estimated response curves. Note also that the Log-Logistic model fit was better (lower AIC) but produces a BMD_{10%} estimate that is twice as large as for the selected model. Because AIC values are so similar, the model average estimate of the BMD_{10%} would be close to the simple average of the estimates in Table D-9 (5.82) which would be a little over 3 times larger than the one proposed.

(iv) Uncertainty factors for kidney and other urogenital system endpoints (Section 2.1.3). English, Marty, Stern

- Is the application of uncertainty factors to the POD scientifically supported and clearly described?

(v) Kidney and other urogenital system-specific reference dose (Section 2.1.4). Bosland, Lash, Rosol

- Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

My comments on model averaging are particularly appropriate for this BMD estimate. From Table D-9

3c. Developmental and reproductive system effects

(i) Developmental and reproductive system hazard (Sections 1.2.3, 1.3.1). Hoberman, Laffan, Meistrich, Persky

- Please comment on whether the available human, animal, and mechanistic studies support the conclusion that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice.
- Are all of the hazards to human development and reproduction adequately discussed?

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- Please comment on the lack of conclusions related to developmental effects as a human hazard of RDX exposure.

(ii) Reproductive system-specific toxicity values (Section 2.1.1). Hoberman, Laffan, Meistrich.

- Is the selection of the [Lish et al. \(1984\)](#) study that describes male reproductive system effects scientifically supported and clearly described?

(iii) Points of departure for reproductive system endpoints (Section 2.1.2). BMD Modeling – Hughes-Oliver, Portier, Stern; HED extrapolation using PBPK modeling – Barton, Morris; HED extrapolation by allometric scaling: Roberts, Stern.

- Is the calculation of a POD for this study scientifically supported and clearly described?
- Are the calculations of HEDs for these studies scientifically supported and clearly described?

Ten (10) models were fit in the process of selecting an estimate for the POD. The process for estimating the POD for reproductive system endpoints follows closely the process for the Kidney/Urogenital system endpoints analysis hence all comments above could be repeated here. The estimated BMD10% is fairly high being between the two highest doses tested suggesting no extrapolation issues. AIC values for the six final models fitted to the data are very close suggesting model averaging might be a good choice here (changes estimate from 56 to approximately 73.8 – not sure how the BMDL10% changes without running the analysis.)

(iv) Uncertainty factors for reproductive system endpoints (Section 2.1.3). English, Marty, Stern.

- Is the application of uncertainty factors to the POD scientifically supported and clearly described?

(v) Reproductive system-specific reference dose (Section 2.1.4). Hoberman, Laffan, Marty, Meistrich

- Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

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3d. Other non-cancer hazards (Sections 1.2.4, 1.2.6, 1.3.1). Buckner, English, Persky.

- Please comment on whether the available human, animal, and mechanistic studies support not drawing any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure.
- Are all of the other non-cancer hazards adequately discussed?

3e. Cancer

(i) Cancer hazard (Sections 1.2.5, 1.3.2). Bosland, Boudrea, Eastman, Klaunig, Persky, Roberts, Rosol; RDX metabolites: Cobb, Turesky

- Please comment on whether the available human, animal, and mechanistic studies support these conclusions that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure (Section 1.3.2).

(ii) Cancer-specific toxicity values (Section 2.3.1). Bosland, Boudrea, Eastman, Klaunig, Portier, Roberts, Rosol

- Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence.

Yes. From Section 1.2.5 we have “The 2-year studies by Lish et al. (1984) and Levine et al. (1983) included comprehensive histopathological examination of major organs, multiple dose groups and a control, and >50 animals/dose group (plus additional interim sacrifice groups). In both studies, the maximum tolerated dose was reached or exceeded in high-dose animals (based on decreased terminal body weight in high-dose male and female mice of 5 and 19%, respectively, and decreased survival in male and female rats by approximately 50 and 25%, respectively, compared to the control).” This suggests well-designed and implemented studies capable of producing the best data for cancer endpoint analysis. Dose-related increases in hepatocellular adenomas/carcinomas and alveolar/bronchiolar adenomas/carcinomas were observed in male and female B6C3F1 mice (Lish et al. 1984). Dose-related increases in hepatocellular carcinomas were observed in male F334 rats (Levine et al. 1983). While EPA's cancer guidelines (U.S. EPA, 2005a) do not require quantitative analysis for substance classified as suggestive of carcinogenic potential the data in Lish et al. (1984) and Levine et al. (1983) are more than adequate to support this analysis. Animal and in vitro studies having lower weight of evidence scores suggest mechanisms for carcinogenicity from RDX exposure and at least two

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MOAs continue to be explored, although lack of adequate toxicokinetic data, especially on RDX metabolites, means that neither of these mechanisms can be confirmed.

- Is the selection of the [Lish et al.](#) (1984) study for this purpose scientifically supported and clearly described?

Yes. Four reasons are given to justify quantitative analysis of liver and lung tumors in female and male B6C3F1 mice from the Lish et al. (1984) study (page 2-25). Of particular note was the recent (2006) reassessment of female mouse liver tissue pathology which updated liver cancer incidences using up-to-date histopathological criteria. Justification was also provided for combining liver and lung benign and malignant tumors for this analysis. Mortality in the mice studies was limited to the highest dose group and was unlikely to significantly impact the quantitative analysis. While dose-response analysis of the F344 male rat liver cancer incidence was also performed, a positive trend was not observed. In addition, the F344 rats in the Levine et al. (1983) study experienced higher mortality at the mid-range of applied doses further complicating and weakening the value of the quantitative analysis.

(iii) Points of departure for cancer endpoints (Section 2.3.2, 2.3.3). Hughes-Oliver, Portier, Roberts, Stern

- Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

Discussion of the BMD₁₀ and BMDL₁₀ calculations are given on pages 2-26 and 2-27 and modeling details are provided in Appendix D section 2. The analysis seems to follow standard guidance using EPA peer-reviewed benchmark dose methodology and software, although it is not clear from EPA BMDS web pages that the MS-COMBO methodology derives from peer-reviewed research or has itself been peer reviewed. Section D.2.2 Modeling Results for Mouse Tumor Data was difficult to follow due to limited narrative. Whereas a 10% ER level is reported in the summary Table (2-7, page 2-28), section D.2.2 shows results, sometimes 10%, sometimes 5% (see for example Table D-17) with the 10% results as a footnote. Everything seems to be there but the presentation is confusing. I am also not certain what it means that each model fitting exercise in this section resulted in choice of the Multistage 1° model.

Calculation of the OSF for hepatocellular and alveolar/bronchiolar adenomas or carcinomas in female B6C3F1 mice is essentially summarized in Table 2-7. The details of OSF calculation are found in the footnote to this table, but then part of this calculation is repeated in the text (pages 2-28 and 2-29).

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I like Section 2.3.4 as concise description of uncertainties related to estimation of the OSF.

4. Dose-response analysis.

4a. Oral reference dose for effects other than cancer (Sections 2.1.5–2.1.8). Hughes-Oliver, Lasley, Miller, Marty, Portier, Stern

- Is the selection of an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the [Crouse et al. \(2006\)](#) study, consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by [Cholakakis et al. \(1980\)](#) that is lower (by approximately fivefold) as described in Section 2.1.4, scientifically supported and clearly described ?

Overall reference dose for nervous system effects scientifically supported: Not my area of expertise.

Overall reference dose for nervous system effects clearly described: Yes (pages 2-16, 2-17)

Overall reference dose for mortality scientifically supported: Report indicates that “In general, this comparison indicates that reference values derived from mortality data would be similar to the final RfD for RDX based on convulsions, assuming the application of the same extrapolation procedures and uncertainty factors.” (page 2-21 line 27) Clearly the scientific support depends on the validity of the extrapolation. Table 2-5 demonstrates concordance of doses at which mortality occurred with doses at which convulsions occurred for the four key rat studies. EPA attempted to model mortality but in only three cases was a model fit successful and in those cases the LD01 estimate was reported similar to the BMD_{10%} for convulsions, although a table showing this comparison is not provided. This seems a difficult and somewhat convoluted comparison and I tend to agree with conclusions although it might be a stretch to say this conclusion is “scientifically supported”. It is a stretch to say the conclusion is evidence-based.

Overall reference dose for mortality clearly described: Yes

Organ/system-specific reference doses scientifically supported: Since nervous system has been discussed separately, this question must refer to Suppurative prostatitis and testicular degeneration. Not my area of expertise.

Organ/system-specific reference doses clearly described: Yes. Both suppurative prostatitis and testicular degeneration reference dose estimates are derived from single studies.

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Reference values are derived from BMD model fits that are clearly documented in Appendix D and show adequate model fits and estimates with adequate properties.

4b. Inhalation reference concentration for effects other than cancer (Section 2.2). Barton, Bruckner, Morris, Persky, Roberts

- Comment on how the available data might support an inhalation reference concentration and describe how one might be derived, given that available studies are considered insufficient to characterize inhalation hazard and conduct dose-response analysis, and that no toxicokinetic studies of RDX are available to support development of a PBPK inhalation model.

4c. Oral slope factor for cancer (Section 2.3.3–2.3.4). Bosland, Boudrea, Eastman, Hughes-Oliver, Klaunig, Portier, Roberts, Rosol, Stern

- Is this derivation of an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice scientifically supported and clearly described?

Isn't this the same question as 3e(iii)?

4d. Inhalation unit risk for cancer (Section 2.4). Barton, Bruckner, Morris, Roberts

- Comment on how the available data might support an inhalation unit risk for cancer given that inhalation carcinogenicity data are not available, nor are toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model, and describe how one might be derived.

5. Executive summary. All Members

- Does the executive summary clearly and adequately present the major conclusions of the assessment?

Missing and should possibly be included is discussion of the concordance in doses producing convulsions and doses at which death occurred in key animal studies.

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Dr. Samba Reddy

3a. Nervous system effects

(i) **Nervous system hazard** (Sections 1.2.1, 1.3.1). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. **Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?**

Prelim. Response:

With regard to nervous system hazard identification, I agree that the available human, animal, and mechanistic studies support the EPA's conclusions that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. Furthermore, I agree that RDX-induced convulsions arise primarily through a rapid mode of action including from RDX-induced GABA-A receptor blockade. However, the evidence presented in the assessment does not fully depict RDX's hazards to the nervous system, that convulsions in rodents can provide a limited spectrum of potential human hazard, and that convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, and neuronal damage can all be part of the spectrum of RDX's potential nervous system hazards. Further evaluation or explanation should be provided for these potential endpoints. In addition, RDX should be considered for the classification as *potential convulsant or proconvulsant to humans*.

(ii) **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the [Crouse et al. \(2006\)](#) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

Prelim. Response:

With regard to nervous system toxicity values, I agree that available studies reporting nervous system effects of RDX are comprehensive and justified. For assessing the convulsant effect, I agree that neurotoxic endpoints, and in particular convulsion endpoints, are appropriate for revealing the hazards of RDX delivered by oral gavage administration (Crouse et al., 20016). However, the relationship between convulsions and mortality is unclear in the overall scheme of assessment of neurotoxicity endpoints for RDX. In addition, we should give more consideration to the data on fatal outcomes and that mortality may arise from non-nervous system factors or hazards.

(iv) **Points of departure for nervous system endpoints** (Section 2.1.2). **Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described?** Are the calculations of PODs for these studies scientifically supported and clearly described? **Is the calculation of the HEDs for these studies scientifically supported and clearly described?** Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the

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benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

Prelim. Response:

With regard to points of departure for neurotoxicity endpoints, I agree that the selection of convulsion as the critical endpoint, especially based on the proposed mode of action of RDX on neuronal GABA-A receptors, is a scientifically acceptable choice to assess the human neuronal hazards of RDX exposure. In addition, I agree that HED are the appropriate basis for extrapolating animal or experimental doses in the assessment of convulsion endpoints. Furthermore, I recommend that we consider the overall spectrum of neuroexcitatory effects from a broader set of the seizure endpoints to fully assess the risk on nervous system. See responses in #3a(i). I suggest that we give more consideration to additional dose data, such as the threshold or median toxic dose on neurotoxicity outcomes of RDX (e.g. ED50 or CD50), including convulsions, seizure threshold to clonic and tonic-clonic seizures and seizure-related mortality. I suggest that we consider potential variables for points of departure for the threshold and convulsion endpoints (e.g. sex differences).

(iv) **Uncertainty factors for nervous system endpoints** (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.2

(v) **Nervous system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

Prelim. Response:

With regard to reference dose for neurotoxicity endpoints, I agree that the dose derived from neurotoxicity assessment, especially for convulsions as the critical endpoint, is scientifically supported but does not entirely depict the dosage for the spectrum of endpoints. Convulsions or seizures represent an "all-or-none" quantal phenomenon, with attributes of a steep dose-response for convulsion endpoints. Therefore, the rationale for not employing the median toxic or convulsant dose (CD50) and associated data (e.g. CD1 or CD10) for causing neurotoxicity incidence of seizures or convulsions is unclear. Like other GABAergic antagonist convulsants (e.g. picrotoxin or pentylenetetrazol), I suggest additional evaluation on dose-response relationship for RDX on the spectrum of neurotoxicity (seizure threshold, seizure discharges, and various convulsions).

Overall, I support the conclusion that the available data in humans and animals support a convulsant or proconvulsant neurotoxicity for RDX, possibly through GABA-A receptor blocking mode of action, and the proposed nervous system-specific reference dose is acceptable. Additional dose specifications should be considered to provide a more dose-response relationship for convulsant and related spectrum of neurotoxic effects of RDX. In addition, RDX should be considered for the classification as *potential convulsant or proconvulsant to humans*.

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Dr. Steve Roberts

3c(iii) Developmental and reproductive system endpoints. Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

Both the POD and the HED for testicular degeneration from the Lish et al. (1984) study are clearly described and scientifically supported. The POD was calculated using Benchmark Dose modeling. BMDL estimates from various models were not “sufficiently close,” and the lowest BMDL was selected, consistent with EPA guidance. Three methods were used to calculate the HED corresponding to the BMDL in mice — one based on allometric scaling ($BW^{3/4}$), another based upon equivalent RDX serum AUCs in mice and humans at steady state, and a third based upon equivalent RDX maximum serum concentrations in mice and humans after a dose. The methods for these calculations are clearly explained on pages 1-10 and C-29 – C-30. Concerns regarding the PBPK model for mice are clearly described on pages 1-9 and 1-10. Given the low confidence in the PBPK model for extrapolating from mice to humans, EPA selected the HED based upon body weight scaling to derive a HED for the testicular degeneration POD.

3e(i) Cancer hazard (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

The case for this classification presented in the document is not strong, in my opinion. It is acknowledged (page 1-74) that an example provided in the 2005 guidelines for “likely to be carcinogenic in humans” fits the data for RDX — “an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans.” There are no examples from the 2005 Guidelines for “suggestive evidence of carcinogenicity” that appear to fit the data for RDX. EPA explains their preference for the “suggestive” classification by saying (page 1-74), “Although the evidence includes dose-related tumor increases in two species, two sexes, and two sites, the evidence of carcinogenicity outside the B6C3F1 mouse is not robust, and this factor was decisive in choosing the hazard descriptor [*suggestive evidence of carcinogenicity*]. Within the spectrum of results covered by the descriptor *suggestive evidence*, the evidence for RDX is strong.” It is not clear to me that RDX evidence in fact fits within the spectrum of results covered by the descriptor

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suggestive evidence as presented in the 2005 Guidelines, or that evidence outside the B6C3F1 mouse needs to be robust in order for the evidence to meet the level of “Likely ...”.

3e(ii) Cancer-specific toxicity values (Section 2.3.1). As noted in EPA's 2005 *Guidelines for Carcinogenic Risk Assessment*, “When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.” Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?”

The rationale is clearly described and taken directly from the quoted passage in the 2005 Guidelines for Carcinogenic Risk Assessment. A case is made why the Lish et al. (1984) bioassay qualifies as a well-conducted study, providing a basis for quantitative dose-response assessment. However, the guidelines language suggests that there are caveats or limitations to uses of cancer-specific toxicity values developed under these circumstances. This document may not be the best venue, but some additional clarification of those limitations, and whether or not they are presented in the IRIS record along with the toxicity value, would be helpful to understand the practical implications of developing a cancer toxicity value for a chemical with “suggestive evidence.”

3e(iii) Points of departure for cancer endpoints (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

The calculation of PODs and oral slope factors (OSFs) is scientifically supported and clearly described. BMDL values were derived for hepatocellular adenomas or carcinomas, alveolar/bronchial adenomas or carcinomas, or a combination of hepatic and alveolar tumors from the Lish et al. (1984) 2-year bioassay of B6C3F1 mice. Given concerns about RDX PBPK modeling to extrapolate from mice to humans (pages 1-9 and 1-10), body weight extrapolation ($BW^{3/4}$) was used. Consistent with EPA guidance for chemicals in which the MOA for tumors is unknown, linear extrapolation from the POD to zero dose is assumed. There is a scientific rationale for developing a cancer potency estimate for liver and lung tumors combined provided that the two are independent. In the absence of a known biological relationship between the two,

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EPA has assumed independence and proposed an OSF based upon combined tumor data. Note: Table 2-8, which summarizes the uncertainty in the derivation of the OSF is a clear and effective tool for articulating key decisions, their effect on the OSF, and the rationale for EPA choices.

4b Inhalation reference concentration for effects other than cancer (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived?

Although some human and animal studies of toxicity from RDX inhalation are available, I concur that individually and collectively these studies are inadequate for development of an RDX RfC. I also agree that toxicokinetic data with which to develop a PBPK model that includes the inhalation route are absent, precluding route-to-route extrapolation.

4c Oral slope factor for cancer (Section 2.3.3 – 2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

See response to 3(iii).

4d Inhalation unit risk for cancer (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

I concur that inhalation carcinogenicity are unavailable, as are inhalation toxicokinetic data with which to construct a PBPK model for the purpose of extrapolating oral carcinogenicity data to the inhalation route. The decision not to develop an inhalation unit risk value for RDX is reasonable and appropriate.

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5 Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Overall, the executive summary is well written. I have no comments or suggestions.

Dr. Thomas Rosol

Question #1: Literature search/study selection and evaluation

The literature search and strategy were well done and thorough and included appropriate sources of literature and studies, including the Department of Defense, Defense Technical Information Center (DTIC) database. Inclusion and exclusion criteria were clearly articulated. Considerations included animal species, sex and strain; experimental design; test article source and exposure; endpoint assessments; and data analysis and presentation.

The quality of the health effects data (human and animal studies) was assessed and reported. Two of three reports with human data were determined to be noninformative.

Experimental animal studies include 3 chronic 2-year studies in mice and rats; 10 subchronic studies in mice, rats, dogs and monkeys; 1 reproductive study in rats; 4 developmental studies in rats and rabbits; and one acute study of nervous system effects in rats. All, except two studies, were only available as unpublished contract research laboratory reports. Studies in nonstandard species (e.g., deer mice, lizards, quails) were not used to assess health effects, but did provide information relevant to toxicokinetics and mechanism of action (MOA).

Question #3b (i): Kidney and other urogenital system hazard (sections 1.2.2, 1.3.1). *The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?*

The two-year bioassay in F344 rats had urogenital effects in the male rats consisting of medullary papillary necrosis, pyelitis, (uremic) mineralization, bladder distension and cystitis, and suppurative prostatitis in the high-dose (40 mg/kg/day) group. Suppurative prostatitis was also reported in the mid-dose groups (1.5 and 8.0 mg/kg/day). Male B6C3F1 mice (320 mg/kg/day) had tubular nephrosis in a 90-day study. Male and female monkeys (10 mg/kg/day) had minimal to mild mineralization of the medulla in the 90-day study. No dose-related changes were found in a subchronic study in dogs or rabbits.

The report emphasized the significance of suppurative prostatitis as a surrogate marker for the urogenital effects in F344 rats and indicated potential relevance to humans. This is somewhat misleading. Prostatitis (nonsuppurative and suppurative) is a common background lesion in rats, particularly F344 rats. The report should distinguish between the incidence of nonsuppurative and suppurative prostatitis and determine if there is a relationship between the two types of prostatitis. The report did not specify what lobes of the prostate gland were affected. It is not clear whether all lobes (dorsal, lateral, ventral, and anterior) of the prostate gland were evaluated. The report also states that the incidence of prostatitis in control rats was low. Suppurative prostatitis in rats is usually due to a low-grade, chronic bacterial infection. Suppurative prostatitis may occur with nonsuppurative prostatitis and both forms of prostatitis can wax and wane in an individual rat. The suppurative prostatitis was associated with the other urogenital lesions

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(papillary necrosis, mineralization, bladder distension and cystitis, and pyelitis), particularly in the high-dose group. Inflammation of the lower urinary tract and urine retention is a predisposing factor for nonsuppurative and suppurative prostatitis. It is likely that the lower urinary tract changes, particularly bladder distension, urine retention, and cystitis, predisposed to the development of suppurative prostatitis.

Suppurative prostatitis was most frequent in moribund high-dose rats and there were only 4 terminal sacrifice high-dose rats with an incidence of 0/4 for suppurative prostatitis. The urogenital lesions were considered a major contributing factor to the moribund state of the rats. It was emphasized that there was a dose-response relationship in the incidence of suppurative prostatitis. The majority of the rats with suppurative prostatitis in the mid-dose groups were also in moribund animals. It would be useful to know if there were any conclusions on the cause of the moribund state in the mid-dose rats, since the other urogenital lesions were not as frequent as the suppurative prostatitis. It is possible that the mid-dose rats also had urine retention, which was not detected as bladder distension, at the time of necropsy.

Prostatitis (nonsuppurative and suppurative) is a common background lesion in rats, particularly F344 rats. The increased incidence of suppurative prostatitis in the mid-dose and high-dose rats was likely secondary to other urogenital RDX-associated findings that contributed to a moribund state. Prostatitis was not reported in other toxicity studies in rats, mice, dogs and monkeys. It was hypothesized that urine retention may have been secondary to the effects of RDX on the GABA_A receptor in the lower urinary tract in rats. This is plausible, but no mechanistic studies support this contention. It is unlikely that the suppurative prostatitis associated with RDX administration will translate to similar findings in other animal species and men; therefore, it should not be considered a surrogate marker for the other urogenital system findings associated with administration of RDX.

Question #3b (ii): Kidney and other urogenital system-specific toxicity values (section 2.1.1). *Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?*

The use of suppurative prostatitis as a surrogate marker of risk assessment of RDX is not justified (see above). Suppurative prostatitis occurred most frequently in moribund F344 rats in the mid-dose and high-dose groups and was infrequent in terminally sacrifice rats. The suppurative prostatitis was likely secondary to other RDX-mediated effects on the urogenital system, particularly in the high-dose group.

Question n #3b (v): Kidney and other urogenital system-specific reference dose (section 2.1.4). *Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?*

Based on comments in 3.b (i and ii) it does not seem appropriate to calculate an oral reference dose based upon the urogenital system findings in the F344 rat 2-year study.

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Question #3e (i): Cancer hazard (sections 1.2.5, 1.3.2). *There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusions.*

The hazard descriptor, 'suggestive evidence of carcinogenic potential' is supported by the animal bioassay data. The incidence of liver tumors (adenomas and carcinomas) was increased in female B6C3F1 mice in a dose-dependent manner and reached statistical significance in some groups (particularly the high-dose group) depending on the analysis. The liver tumor incidence data were variable in male mice and were not convincing for a RDX-mediated effect. A review of the liver tumors in female mice was conducted by a Pathology Working Group (PWG), which provided additional validity to the interpretation and (re)classification of adenomas and carcinomas based on current standards. The lung tumor data in B6C3F1 male and female mice were less supportive of a carcinogenic effect; however, statistically significant increases in tumor incidence were reported for carcinomas or combined adenoma/carcinoma. The data on liver tumors in F344 rats did not support a carcinogenic effect of RDX.

Question #3e (ii): Cancer-specific toxicity values (section 2.3.1). *As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?*

The rationale for quantitative analysis of carcinogen risk analysis was appropriately justified and the selection of Lish et al (1984) was supported and clearly described.

Question #4c: Oral slope factor for cancer (sections 2.3.3 – 2.3.4). *The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?*

The lung tumor data is not considered robust, so it may not be advisable to combine the mouse liver and lung tumor data for analysis of the oral slope factor.

Question #5: Executive Summary. *Does the executive summary clearly and adequately present the major conclusions of the assessment?*

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There is too much emphasis on the incidence and significance of the suppurative prostatitis. The other urogenital system effects are of more importance and should be described. It does not seem appropriate to calculate a reference dose based on the incidence of suppurative prostatitis.

Dr. Alan Stern

1. Literature search/study selection and evaluation.

The section on Literature Search Strategy / Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search strategy was appropriate and clearly described. Figure 15-1 and Table 15-1 were well constructed and informative. However, I did not find the material presented starting on pg. xxxiii onward to be necessary or particularly helpful. The Selection Criteria section is largely a restatement of basic risk assessment principles that are found elsewhere in IRIS guidance, or a summary of the decision process specific to RDX that is found in greater detail in the body of the document.

*3a. (iii) **[BMD modeling]***

Points of departure for nervous system endpoints (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

The benchmark dose modeling results are clearly reported in the Supplemental Material and the information presented is inclusive of the data necessary to justify the model selection. In the document, the EPA chose to use a BMR of 1%. This is unusual. The USEPA benchmark dose modeling guidance (https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf) gives a default BMR for quantal data of 10%, but notes that various factors can justify moving off this default. Among these factors, the guidance states that, “*Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects (e.g., frank effects), or a BMR greater than 10% (e.g., for early precursor effects) as the basis of a POD for a reference value.*” The justification in the document (although not stated in these specific terms) is that convulsions, and particularly convulsions possibly associated with mortality, are a “frank” effect. However, the choice of the BMR should also be

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based on the distribution of the dose response data such that the BMR should be within, or close to the lower range of the reported response. In this respect, the benchmark dose guidance also states that, "...if one models below the observable range, one needs to be mindful that the degree of uncertainty in the estimates increases. In such cases, the BMD and BMDL can be compared for excessive divergence. In addition, model uncertainty increases below the range of data." In the case of convulsions for M and Frats -combined, the response at the lowest dose giving an observed incidence of convulsions (8 mg/kg/d) was 15%. While this response level can reasonably justify a BMR of 10%, a BMR of 1% appears difficult to justify in terms of the extent of extrapolation required. In comparing the BMD and BMDL for the selected model (as suggested by the BMD guidance), the BMR of 3.02 mg/kg/d is about 5 times the BMDL of 0.569 mg/kg/d. The document does not present this comparison. In choosing a BMR of 1%, EPA appears to have been driven by concerns about frank effects, but does not appear to have taken the data-based requirements of the benchmark dose modeling into account. This needs to be more fully discussed in the document.

3a. (iv) Uncertainty factors for nervous system endpoints (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.

The UF for sensitive human populations (UF_H) is appropriate, This is also the case for the $UF = 3$ for average animal to average human (UF_A) given the $bw^{3/4}$ toxicokinetic scaling and the lack of needed adjustment ($UF = 1$) for a LOAEL to a NOAEL because of benchmark dose modeling.

For the UF for subchronic duration (UF_S), EPA's justification for a value of 1 rests, in part, on the lower dose threshold for effects in subchronic studies compared to chronic studies. The explanation for this somewhat counterintuitive observation is that the gavage dosing in subchronic studies resulted in greater peak doses to the nervous system receptors than the dietary exposures in the chronic studies. While this explanation is reasonable, it doesn't actually address the appropriate (but unavailable) comparison between threshold for convulsions in subchronic dietary exposures and chronic dietary exposures. The other basis for EPA's justification for the value of 1 for this UF is that neurologic effects of RDX appear to be more related to dose than to duration. However, in its discussion of its choice of AUC versus peak blood concentration, (pg. 2-9), EPA makes the argument that, "*There is evidence from examination of picrotoxin binding to GABA_A that a resulting period of elevated neuronal activity post-exposure could result in increased likelihood of seizure developing over time or other the longer-term effects on normal brain function.*" This suggests that EPA believes that duration of exposure (i.e., the cumulative dose) could also result in increased sensitivity. Thus, it is not clear that EPA has adequately

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justified a UF of 1 (i.e., no adjustment for the subchronic duration of the critical study). While it is not obvious that a UF of 1 is inappropriate, EPA should address these two issues.

The uncertainties EPA identifies in the studies of neurologic effects (incomplete observation of animals post-dosing, lack of ascertainment of “upstream” neurologic effects) are valid and appropriate and warrant an adjustment by a factor of 3. I would suggest, however, as a matter of formal structure, that this is not strictly a dataset insufficiency, since, in my experience, this category of UF is applied when categories of studies (e.g., reproductive) are missing, not necessarily when the studies in a given category are less than ideal. Perhaps the better category for this UF of 3 would be as a modifying factor (UF_M).

3b. (iii) BMD modeling

Points of departure for kidney and other urogenital system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described?

Based on the information presented in the Supplemental material (Appendix D), the benchmark dose modeling of the suppurative prostatitis appears to be reasonable. The BMR of 10% is standard and appropriate here. It should be noted that the dose-response curve is quite steep in the range of the BMD and this leads to some instability in the identification of the appropriate BMDL. Nonetheless, the BMDL from the log-probit model is the most appropriate choice for the POD.

3b. (iv) *Uncertainty factors for kidney and other urogenital system endpoints (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?*

The same suite of uncertainty factors was applied to all endpoints. This is appropriate the UF_H, UF_A, and UF_L factors (as discussed above (3a, iv)). However, the case for use of a UF_S (subchronic) of 1 appears to rest on issues of neurologic effects and in particular on the timing of convulsions with gavage versus dietary routes of exposure. EPA does not appear to make an argument as to why such considerations should apply to non-neurological (in this case, urogenital) endpoints. Likewise, it is not clear why the UF_D (database uncertainty) that is justified on the basis of insufficient data to address neurologic effects “upstream” from convulsions should apply to non-neurological (e.g., urogenital) endpoints.

3c. (iii) BMD modeling

Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described?

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The benchmark dose modeling for testicular degeneration is appropriate and clearly described (in Appendix D). The selected models both give acceptable fits and also yield the lowest BMDLs for each of these endpoints. In addition, the use of a BMR of 10% is consistent with the data, as the LOAEL occurs with a responses almost exactly at 10%.

[Allometric scaling]

Is the calculation of the HED for this study scientifically supported and clearly described?

Given the instability in the mouse PBPK model, estimation of the HED using $bw^{3/4}$ scaling is appropriate and is clearly described.

3c. (iv) Uncertainty factors for reproductive system endpoints (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

The issue here for male reproductive endpoints is the same as that described above (3b. iv). It is not clear why the UF_S and UF_D that are justified on the basis of considerations that appear specific to neurological effects are appropriate for male reproductive effects.

3e. (iii) Points of departure for cancer endpoints (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

EPA combined tumor incidence from multiple sites in the mouse for benchmark dose modeling and derivation of the POD. Although in the teleconference, EPA stated that this was its standard operating procedure, this is not clear to me from my experience. At a minimum, EPA should discuss this policy and give examples of where this choice was and wasn't made in other cancer risk assessments.

The use of the MS-COMBO approach to combining independent tumors incidence from multiple sites (pg. D-31, lines 19-22) is not clearly described. It is not clear how this approach differs from simply summing total tumor incidence at each dose and using these incidences for benchmark dose modeling.

EPA conducted the benchmark dose modeling using only the multistage model as a matter of policy. Visual examination of the plot of the fit of the multistage model to the combined mouse hepatocellular and respiratory tumors in female mice shows that, although the multistage model (1^0) gave an acceptable fit to the data, other models are likely to have given better fits. Since use of the multistage model as a default for cancer dose-response modeling is a theoretically-based default, it is not clear to me why empirical data (i.e., the fit of alternative models) shouldn't trump the theoretical construct. EPA should discuss this decision and comment on its implications.

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4a. Oral reference dose for effects other than cancer (Sections 2.1.5–2.1.8). The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on 6 nervous system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

For lethality from neuro-excitation, it is not clear that benchmark dose modeling is appropriate for attempting to elucidate the difference in dose-response between convulsions and lethality since there is probably a sharp threshold for lethality and extrapolation of the dose-response with the fitted models – even slightly beyond the observed data – may obscure this threshold.

The discussion of the comparison of the LD₀₁ for lethality from Crouse et al. (2006) to the distribution of LD₀₁ values from the other relevant studies is a critical consideration and would be helped by a graphic.

The notion of confidence intervals (CIs) for NOAELs for lethality (starting on pg. 2-21, line 17) is presented without any explanation. A short (one sentence) introduction would be helpful here.

Since NOAELs are study-specific, the utility of comparing NOAELs across studies is not clear. Furthermore, as discussed in my previous comment, the likelihood of a sharp threshold for lethality makes the use of CIs for NOAELs additionally problematic.

The conclusion that reference values for mortality would be similar to RfDs for convulsions (pg. 2-21, lines 26-30) was obvious from Table 2-6. I don't think that the analysis on pg. 2-21 added anything useful to that and could be deleted.

With respect to consideration of the lower RfD derived from Cholakakis et al. (1980), the arguments about the better design, execution and greater purity of the test material in the Crouse et al. (2006) study versus the Cholakakis et al. (1980) study notwithstanding, Cholakakis et al. (1980) did produce a much lower POD_{HED} (0.06 vs. 0.28 mg/kg/d), as well as a lower LOAEL (0.2 vs. 8 mg/kg/d). Furthermore, the endpoint is clear cut and easily interpretable and (as described in the text) it is closely linked to mortality. The argument presented in the text that Cholakakis et al. (1980) was not specifically designed to assess neurological effects and therefore, monitoring of convulsions was incomplete, only makes the report of those convulsions that *were* observed that much more meaningful. The difference in RfDs and LOAELs between these two studies appears to be meaningful unless concrete reasons are presented to consider the Cholakakis et al. essentially invalid (i.e., that the low-dose convulsions that were observed are either incorrectly reported or suspect due to the nature of the material).

4c. Oral slope factor for cancer (Section 2.3.3–2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

The technical operations involved in the calculation of the oral slope factor (OSF) appear correctly and appropriately done and clearly reported (if the Supplemental material is also consulted).

Table 2-8, the summary of uncertainties in the derivation of the OSF, is a good idea and provides a well-presented guide to the decision process involved in the derivation. I have several comments relating to the information presented in this table.

- Combined Tumor Types – The combination of tumor types in the calculation of the OSF is justified (in part) by EPA by the lack of any known “biological dependence” of these tumor types. This term is not defined and is not intuitively obvious to me. Clearly, there is some biological dependence among these tumor types since they are all caused by RDX exposure. Thus, they may share a mode of action (MOA). Does “dependence,” therefore, refer to commonality in tissue source (i.e., appearance of tumors in different tissues as the result of metastasis)? It is also not clear what approach would be used, or how it should differ from the presented approach if the tumors types *were*, in fact, “dependent” (under some definition of that term).
- BMD Modeling Uncertainty, Alternate (BMD) model selection – As discussed in my response to charge question 3e. iii, because, as stated in Table 2-8, no biologically-based dose-response models for RDX are available (including the multistage model), it is not clear why the multistage model was exclusively investigated for BMD cancer dose-response modeling. Although there is *some* theoretical basis for use of the multistage model, this relates only to the multiple mathematical forms that the model can apply to the data (e.g., 0, 1st, 2nd, etc degree functions). However, these individual mathematical forms within the multistage model do not, themselves, have any biological significance. Thus, it is unclear to me why the multistage model should have exclusive claim to cancer dose-response modeling even if other (non-biologically-based) models provide a better empirical fit to the data.

5. Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

In the brief discussion of neurologic effects within the section, “Effects other than cancer observed during oral exposure,” there is no mention of the lethality associated with convulsions.

In the section on “Suppurative prostatitis,” the possibility of a bacterial infection is raised and its potential significance to RDX toxicity is briefly discussed. However, it should also be noted that

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(as I understand it) this effect could also be secondary to inflammation without a bacterial infection.

The Executive Summary does not provide any kind of summary statement addressing the confidence in the RfD or

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Dr. Robert Turesky

Hazard identification and dose-response assessment.

3e. Cancer

(i) Cancer hazard (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

Preliminary evaluation: *The published data provides insufficient information to assess the carcinogenic potential of RDX in humans. The mode of action of RDX, its genotoxicity, and mechanism of carcinogenicity in rodents cannot be evaluated with the current published literature data, which are very sparse. The evidence for RDX carcinogenicity includes dose-related tumor increases in two species, two sexes, and two sites. However, the evidence of carcinogenicity in rodent models other than the B6C3F1 mouse is weak, and the mode of carcinogenic action of RDX in rodents cannot be determined based on our current understanding of RDX metabolism. Therefore, descriptor of suggestive evidence of carcinogenic potential is appropriate.*

Comments on the RDX Draft Report.

On Page 110 (1-69), lines 26 – 27 of the Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine it is stated “demonstrated some genotoxic potential. While these metabolites have been measured in the mouse (Pan et al., 2007bMusick et al., 2010) and minipig (Major et al., 2007), they have not been identified in humans, and may not be the predominant metabolites of RDX. A MOA involving a proliferative response generated by tissue-derived oxidative metabolites of RDX has been proposed, but is not supported by the available data”

Please note, the formation of many carcinogenic metabolites of procarcinogens (or their solvolysis products) in rodents or humans are often the minor components. Therefore, the fact these compounds are formed in minor amounts does not dismiss their formation as potential mechanism for the genotoxic effects of RDX in rodents.

In the Supporting Report it is stated on page C-8 and also in the heading of Table C-2 that 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide are principal terminal urinary metabolites - These statements are inaccurate. Both compounds represent <2% of the dose. They are minor metabolites (or they underwent further biotransformation prior to elimination in urine). The identities of the majority of the 14C associate material in urine are unknown.

Comments on Mode of Action of RDX and Cancer.

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Publications critical to the mode of action of RDX as a genotoxic or nongenotoxic carcinogen.

There are no data published characterizing the metabolism of RDX with rodent or human liver or lung enzymes or the metabolism of RDX by rodent and human bacterial flora. There are no animal studies demonstrating oxidative stress or tumor promotion by RDX. The published data are inadequate to provide an explanation of mechanisms and the mode of carcinogenicity of RDX.

The genotoxicity and mammalian cell toxicity data are limited. Snodgrass (1984) assessed genotoxicity of MNX in the Ames test, mouse lymphoma forward mutation assay, cytogenetic assays in CHO cells, unscheduled DNA synthesis in primary rat hepatocytes, and dominant lethal effects in mice. The chemical purity of test compound was not reported. No activity was observed in the Ames test; MDX induced mutant frequency at TK locus with and without S-9 (Arochlor-induced S9 rat liver); doses and dose response curves were not reported, and differences of mutant frequencies with and without S9 activation, were not reported. Chromosome aberrations were reported in CHO cells in the presence of S9 activation but not without, but doses were not reported. MDX induced DNA repair (³H-thymidine nuclear labeling) in rat hepatocytes but doses of MDX were not reported. The chemical purity of test compound was not reported.

Bacterial mutagenesis in the Ames reversion assay is largely negative except for the study by Pan et al., 2007b. The EPA report states that the study employed high levels of rat-liver S9 to activate RDX derivatives. However, many N-nitroso compounds are weakly positive or do not elicit effects in Ames assays, which in part may be attributed to their high reactivity and solvolysis prior to entry into the bacterial cell. (M. Cooper, Mutation Research 454 (2000) 45–52; H. Kushida et al., Mutation Research 471 (2000) 135–143) Also, note that Kamataki and colleagues did use 20% S-9 prepared from phenobarbital- and 5,6-benzo-flavone-treated rat in the bacterial mutagenesis assays for N-Nitrosodiethylamine (NDEA), and other N-nitroso compounds (H. Kushida et al., Mutation Research 471 (2000) 135–143O). None of the reported mutagenicity assays on RDX had used known carcinogenic N-nitroso compounds as positive controls, when assaying RDX or its nitroso derivatives. Such studies would provide additional information about relative bacterial mutagenic potencies of RDX and its N-nitroso compounds to known N-nitroso carcinogens. The Cooper and Kushida papers report the use of Ames tester strains expressing specific P450s known to bioactivate N-nitroso compounds. Such tester strains should be employed to assess the genotoxicity of RDX and its nitroso compounds.

The literature data suggest that bacteria in the soil, when cultured in vitro, can metabolize and denitrify RDX, to produce ring cleavage products and the generation of CO₂, NH₃ and formaldehyde; there may be transient formation of nitroso intermediates that further degrade into alkylhydrazines as minor products; these compounds are genotoxic and/or carcinogenic. However, the extent of formation of these hydrazine derivatives in soil and their persistence are unknown. Most biotransformation studies on RDX were conducted in culture and may not occur in the soil or water, or the metabolic products may rapidly decompose. There has been no report on the detection of hydrazine metabolites formed in vivo in rodent studies, or if such product formation is catalyzed by fecal flora of rodents or humans, or if these hydrazine metabolites are produced in rodent models and induce DNA damage. Notably, RDX appears to be highly

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bioavailable in rodents: oral bioavailability of ^{14}C -RDX in rats is >85% and >50% in mice. 4-Nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide were identified as minor metabolites in the minipig model (Major et al., 2007). The biochemical toxicology and mutagenesis studies have not been examined for either compound and investigations are clearly warranted.

Metabolic studies have not been conducted on RDX with human hepatocytes, liver extracts, recombinant cytochrome P450s, or bacterial flora of the human gut, or in vivo. Data regarding the formation of genotoxic, DNA damaging metabolites of RDX in vitro or in vivo in humans do not exist. There are no human or rodent enzymes known to catalyze the bioactivation of RDX, or established biomarkers to provide support for a role of DNA adduct formation or oxidative stress as a mechanism of DNA damage by RDX derivatives, as has been reported for other nitro compounds such as trinitrotoluene or nitropropane, or nitroso compounds, i.e. dimethylnitrosamine, nitrosopiperazine, etc.

Summary of relevant studies

Bacterial mutagenicity:

S.E. George et al. / *Mutation Research* 490 (2001) 45–56

George et al assayed the *Salmonella tryphimurium* reverse mutation assayed RDX derivatives in Ames reversion assay. RDX gave a negative response in strains TA98 and TA100 (up to 250 $\mu\text{g}/\text{plate}$) which is consistent with earlier reports. Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3-nitroso-5-nitro-1,3,5-triazine (DNX) were not mutagenic; however, hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), was weakly mutagenic in TA100 (0.3 rev/ μg test compound) with and without activation by S-9, but not mutagenic in TA98. Note, similar activity was observed with and without S-9 bioactivation (the source of S9 animal tissue and pretreatment chemical that induces P450 enzymes, such as PCB or 3-MC was not provided). A dose response curve was not reported.

The mutagenicity of TNX was far weaker than that reported for 2,4,6-trinitrotoluene (without metabolic activation) in TA98 and TA100. TNT and its DNT derivatives show much higher mutagenic potencies in YG1041 (TA98 derivative) and YG1042 (TA100) derivative), strains that overproduce both *O*-acetyltransferase and nitroreductase, the mutagenic potency of 24DNT, 26DNT, and their metabolites was higher than in the parental strains. (M. Sayama et al. *Mutation Research* 420 (1998) 27–32) These findings are consistent with the formation of an arylhydroxylamine metabolite, which subsequently undergoes *O*-acetylation to form reactive *N*-acetoxy-arylamines that react with DNA via the nitrenium ion (SN_1) or through nucleophilic displacement (SN_2) mechanism. The biochemical mechanism of putative adduct formation for RDX derivatives, which are not aromatic substituted nitro compounds, is quite different from TNT derivatives. Genotoxic *N*-nitroso compounds of secondary amines undergo bioactivation by α -hydroxylation. The formation of such reactive metabolites of RDX has not been shown.

Pan et al *Mutation Research* 629 (2007) 64–69

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Pan et al assayed the mutagenicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and its *N*-nitroso derivatives hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) in the *Salmonella tryphimurium* reverse mutation assay (Ames assay) with strains TA97a, TA98, TA100, and TA102. Using a preincubation procedure and high S9 activation (9%. Rat liver from MOLTOX). The tester strain TA97a, was more sensitive than TA98, TA100, of TA102. TA97a is a frame-shift tester strain with mutations occurring at a run of C and G-C base pairs. A relatively large quantity of rat liver S-9 was employed in the assay and required for mutagenesis. Weak mutagenic effects were observed at the highest dose level with a mutagenicity index (MI) of about 3.5 for TNX (1120 µg/plate), ~ 3.0 for MNX (878 µg/plate), and ~ 2.0 for RDX (1090 µg/plate). *N*-nitroso compounds (NOC) require metabolism at the carbon atom α to the *N*-nitroso group to generate reactive electrophiles that can form DNA adducts. NOC are generally weak bacterial mutagens, which may be attributed to their high reactivity and decomposition/solvolysis prior to reaching DNA within the bacteria. The reactive intermediates of RDX derivatives that form DNA adducts and induce mutations is not known.

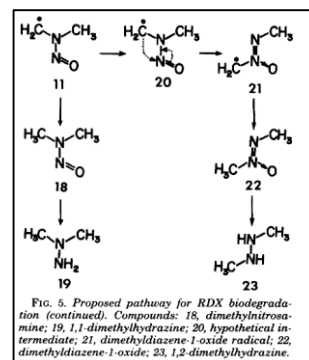
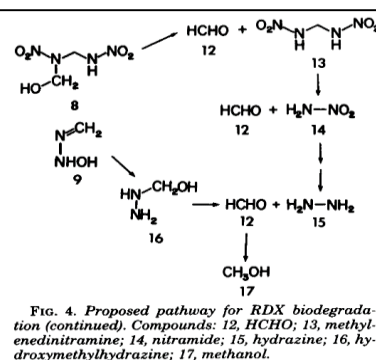
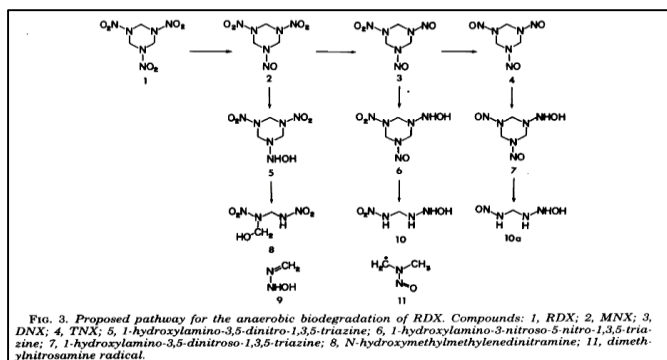
Bacterial metabolism and bioactivation of RDX:

Zhao et al., Can. J. Microbiol. 50: 91.96 (2004)

The metabolism of RDX was evaluated anaerobically at 10 °C with marine sediment from a previous military dumping site of unexploded ordnance. The disappearance of RDX was accompanied by the formation of the mononitroso derivative MNX, which appeared transiently, and by formation of formaldehyde (HCHO) that subsequently disappeared. No di- or tri-nitroso derivatives of RDX were detected.

N. G. McCormick et al. Applied and Environmental Microbiology, Vol 42, 817-823 (1981)

Biodegradation of [¹⁴C]-labelled RDX under anaerobic conditions from nutrient broth cultures inoculated with anaerobic sewage sludge yielded a number of products, including: MNX, DNX, TNX, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, formaldehyde, and methanol. The presence of carcinogenic hydrazines was supported by GC/MS data. Note the overall % conversion to the genotoxic hydrazine derivatives was not reported. The proposed formation of these reactive intermediates are depicted from Figs. 3 – 5 of their manuscript. Both 1,1- and 1,2-dimethylhydrazine and their immediate precursors,

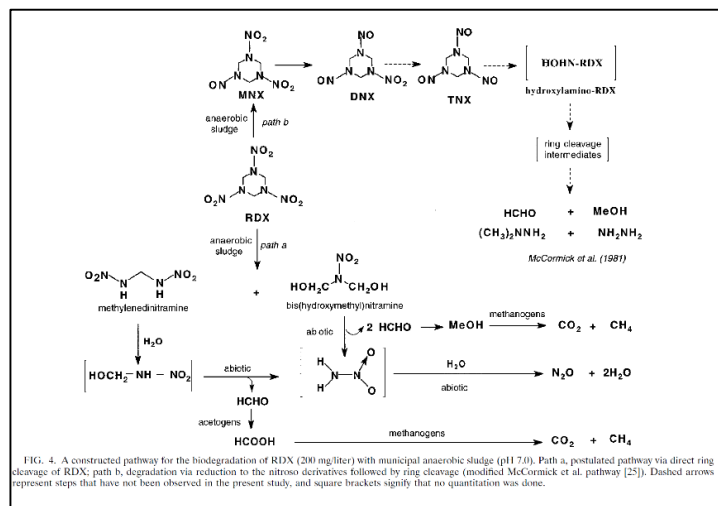


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dimethylnitrosamine and azoxymethane, as well as hydrazine, are known mutagens or carcinogens or both. The amounts of these compounds formed in the soil, chemical stability and biological fate are not known (verify this).

J. Hawari et al. Applied and Environmental Microbiology, Vol 66, 2652 – 2657 (2000)

Hawari et al. characterized the degradation of RDX in liquid cultures with municipal sludge. They proposed two routes of metabolism. The first route involved the production of the nitroso derivatives MNX and DNX. The second route produced two presumed ring cleavage products methylenedinitramine and bis(hydroxymethyl)nitramine, similar the pathway reported by McCormick. None of the metabolites persisted in the incubation system, disappearing, to give rise to nitrous oxide, formaldehyde, methanol and formic acid which in turn was broken down to produce methane and carbon dioxide. The two degradation pathways are shown in Figure 4 of their manuscript. There is a potential to form genotoxic *N*-nitroso compounds from these intermediates.

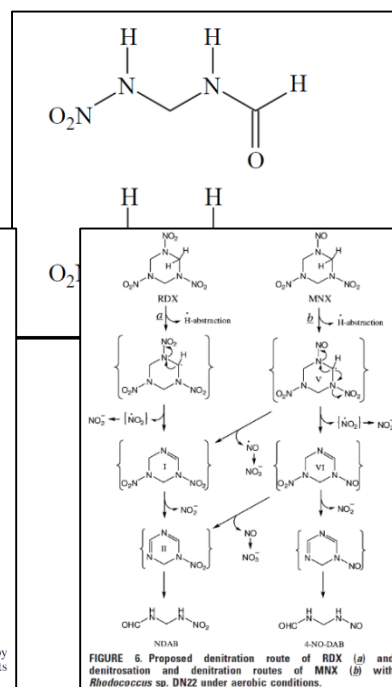
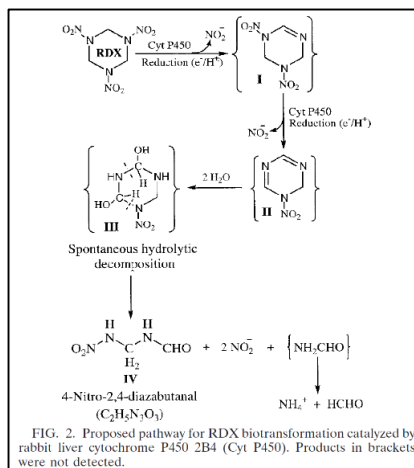


Mammalian metabolism and bioactivation of RDX

Michael A. Major et al. Metabolite Profiling of [¹⁴C]hexahydro-1, 3, 5-trinitro-1,3,5-triazine (RDX) in Yucatan Miniature Pigs', Journal of Toxicology and Environmental Health, Part A, 70: 14, 1191 — 1202

¹⁴C-RDX, (43 mg/kg) was given as an oral dose (carboxymethyl cellulose suspension) to minipigs. 4-Nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide were observed as minor metabolites excreted in urine (<1% of dose). These metabolites possibly could be converted to reactive intermediates that damage DNA. Studies have not been conducted to assess DNA damaging and genotoxic potential of these compounds in bacterial assays.

Their identities were determined by LC-MS/MS. Trace levels of unmetabolized RDX was also detected. Analysis by LC-MS/MS also characterized trace amounts of MNX (in both male and female urine) and DNX (in male urine). Analysis of plasma by LC-MS/MS also revealed low but quantifiable levels of RDX,



and MNX, DNX, and TNX. At 24 h post-dose, none of the liver extracts showed quantifiable levels of RDX or any identifiable metabolites. Most of the radioactivity appeared in the form of water soluble high-molecular-weight compounds. Given that $^{14}\text{CO}_2$ is formed, it is uncertain whether the high molecular weight products are derived from reactive intermediates of RDX that bind to macromolecules or if the ^{14}C -labelled material is attributed to the metabolic incorporation of $^{14}\text{CO}_2$ into lipids, proteins, carbohydrates, DNA or RNA. The data reveal that RDX when given orally to pigs is rapidly metabolized by loss of two nitro groups followed by ring cleavage.

B. Bhushan et al. *Applied and Environmental Microbiology*, Vol 69, p. 1347–1351.

Bhushan reported that RDX biotransformation by rabbit cytochrome P450 produces 4-nitro-2,4-diazabutanal, nitrite, formaldehyde, and ammonia. Their proposed mechanism of formation is depicted in the following figure. Subsequently, they showed that the *Rhodococcus* genus, of aerobic, nonsporulating, nonmotile Gram-positive bacteria, can produce 4-nitro-2,4-diazabutanal (*Environ. Sci. Technol.* 2010, 44, 9330–9336).

Snodgrass (1984) assessed genotoxicity of MNX in the Ames test, mouse lymphoma forward mutation assay, cytogenicity assay in CHO cells, unscheduled DNA synthesis in primary rat hepatocytes, and dominant lethal effects in mice. Note: incomplete information provided in Ames tester strains, dose of compound and % purity of MNX. No activity in Ames test; MDX induced mutant frequency at TK locus with and without S-9 (rat Arochlor S9, Arochlor induced); doses were not reported and differences of mutant frequencies with and without S9 activation were not reported. Chromosome aberrations were reported in CHO cells in the presence of S9 activation but not without. Doses were not reported. MDX induced DNA repair (nuclear labeling) but doses of MDX were not reported. MDX was negative in dominant lethal test in mice (doses not reported).

Carcinogenic potential of RDX in rodents:

The female B6C3F1 was most sensitive to the carcinogenic effects of RDX, with increased incidence of hepatocellular neoplasms when RDX was given as part of the diet. (Lish et al, 1984). The tumor incidence in concurrent female control mice was relatively low (1/65), and significantly lower than incidences of historical control data (note, those data were not provided). The combined incidence of hepatocellular adenomas or carcinomas in female mice at RDX 7 doses ≥ 35 mg/kg-day (19% at both doses) was statistically significantly elevated when statistical analysis was performed using NTP historical control data. The EPA report on RDX provides the control data incidence for female mice of the same strain from National Toxicology Program (NTP) studies conducted during the same time period (147/1,781 or 8%; range: 0–20%). EPA noted caution in extrapolation of negative control data not concurrently conducted because of cross-study differences in labs, diets, and sources of animals. The carcinogenicity data was reevaluated by Parker et al, and reported 2005. The Pathology Working Group (PWG) members reviewed the histologic sections without knowledge of treatment group or previous diagnosis by the study pathologist or the reviewing pathologist. The PWG reported a slightly lower incidence of neoplasms at each of the dose levels. The decreased incidences reported for neoplasms was due to

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reclassification of hepatocellular adenomas as foci of cytoplasmic alteration, in compliance with more current diagnostic criteria. The increased incidence of hepatocellular neoplasms in female mice given RDX at 35 mg/kg/day was interpreted as equivocal evidence of a carcinogenic effect. In male mice, the incidences of combined hepatocellular adenomas or carcinomas were higher in three of four treated groups than in the control, but there were no statistically significant trends for either case.

Lung tumors reported as alveolar/bronchiolar/adenomas or carcinoma combined were reported to increase in both male and female B6C3F1 mice; however, authors regarded the neoplasms as random and not biologically significant (Lish et al. 1984). The induction alveolar/bronchiolar carcinoma incidence showed a weak statistically significant trend ($p < 0.05$) was identified using a one-sided Cochran-Armitage trend test. The incidence over controls was 1.66 fold in males and 1.74 in females at the 35 mg/kg/day dose.

The Hart study used Sprague-Dawley rats at 0, 1, 3.1 or 10 mg/kg-day with no evidence of increased hepatocellular adenoma or carcinomas in either sex, but doses were lower than those given in mice of the Lish study (maximum tolerated dose at 35 mg/kg-day). A study by Levine showed positive trend in hepatocellular carcinoma in male but not females rats (Fisher 344) but there were only a few tumors observed in the control group, and the combined neoplastic nodules and hepatocellular carcinoma was not significant in treated vs control animals. Bioassays did not show incidence of lung tumors at doses up to 40 mg/kg-day.